ESTCP
Cost and Performance Report

(ER-200517)

The Effect of Soil Properties on Metal Bioavailability: Field Scale Validation to Support Regulatory Acceptance

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TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

ACRONYMS AND ABBREVIATIONS

ACS	American Chemical Society
AFB	Air Force Base
Al	aluminum
Alox	Aluminum Reactive Oxide Fraction
APS	Advanced Photon Source
As	arsenic
BAF	bioaccumulation factor
BARGE	Bioavailability Research Group of Europe
C	carbon
Ca	calcium
$CaCl_2$	calcium chloride
$Ca(NO_3)_2$	calcium nitrate
CBD Fe	citrate-bicarbonate-dithionite extractable iron
Cd	cadmium
CEC	Cation Exchange Capacity
Cr	chromium
Cu	copper
DL	Detection Limit
DoD	U.S. Department of Defense
DOE	U.S. Department of Energy
EC	electrical conductivity
EcoSSLs	ecological soil screening levels
ERA	ecological risk assessment
ESTCP	Environmental Security Technology Certification Program
EXAFS	Extended X-Ray Absorption Fine Structure
Fe	iron
FEAL	amorphous iron and aluminum oxides
Feox	iron reactive oxide fraction
GI	gastrointestinal
HCl	hydrochloride
Hg	mercury
HQ	hazard quotient
ICP-AES	inductively coupled plasma atomic emission spectroscopy
IDW	investigation derived waste
IVBA	*In vitro* Bioaccessibility

ACRONYMS AND ABBREVIATIONS (continued)

IVG	In vitro Gastointestinal
µm	micromole
M	molar
MCAS	Marine Corp Air Station
mL	milliliter
MLR	multiple linear regression
Mn	manganese
Mnox	manganese reactive oxide fraction
NaCl	sodium chloride
NaOH	sodium hydroxide
Ni	nickel
NCEA	National Center for Environmental Assessment
NRC	National Research Council
NSA	Naval Support Activity
NSCRC	Natural Science and Engineering Research Council
ORNL	Oak Ridge National Laboratory
OSU IVG	Ohio State University *In vitro* Gastrointestinal Method
Pb	lead
PBET	Physiologically Based Extraction Test
PbS	lead sulfide
PNC-CAT	Pacific Northwest Consortium Collaborative Access Team
PNC-XOR	Pacific Northwest Consortium/X-ray operations and research
PRESS	predicte residual sum of squares
RBA	relative bioaccessibility/bioavailability
RBALP	relative bioaccessibility leaching procedure
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
RMSE	Root Mean Square Error
rpm	revolutions per minute
RR	ridge regression
SBAT	Soil BioAccessibility Tool
SBET	Simplified Bioaccessibility Extraction Test
SBRC	Solubility Bioavailability Research Consortium
SERDP	Strategic Environmental Research and Development Program
SOP	Standard Operating Procedure
SSRL	Stanford Synchrotron Radiation Laboratory

TRV	toxicity reference value
UEF	Urinary Excretion Fraction
USEPA	U.S. Environmental Protection Agency
XANES	x-ray Absorption near-edge structure
XAS	x-ray absorption spectroscopy
XRF	x-ray fluorescence
Zn	zinc

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EXECUTIVE SUMMARY

The U.S. Department of Defense (DoD) faces a potentially daunting task of remediating thousands of metal-contaminated sites within the U.S. and its territories that contain unacceptable levels of the toxic metal(loid)s arsenic (As), cadmium (Cd), chromium (Cr), and lead (Pb). With the exception of Pb contaminated soils, human health and ecological risk drivers have prompted the U.S. Environmental Protection Agency (EPA) to assume that the total soil metal concentration is 100% bioavailable. Previous Strategic Environmental Research and Development Program (SERDP) funded research (ER-1166) has shown that the ubiquitous metal-sequestering properties of soil can significantly lower the bioavailability and risk of heavy metals to human and ecological receptors. This investigation brought together regulators, EPA, end-users, and scientists to demonstrate the applicability of these concepts by showing that simple, readily available soil properties can often be used to predict the bioavailability of As, Cd, Cr, and Pb with a reasonable level of confidence. We have shown that *in vitro* methods can often be used for risk assessment of toxic metals in soil by comparing *in vitro* and *in vivo* metal bioavailability studies.

OBJECTIVES OF THE DEMONSTRATION

The technical objectives of the investigation were: (1) To provide validation that the relationships between soil properties and *in vitro* bioaccessibility methods can serve as a screening tool for estimating *in vivo* toxic metal bioavailability in DoD soils; (2) To provide DoD with a scientifically and technically sound method for estimating human and ecological risk associated with metal contaminated soils in place of or as justification for more-detailed, site-specific bioavailability (e.g., animal dosing), and (3) to promote the use of *in vitro* methods in human health and ecological risk assessments through the upfront involvement of end-users and regulators and the subsequent dissemination of the results of the study in peer-reviewed journals.

TECHNOLOGY DESCRIPTION

Metal Speciation

- Characterize the molecular-level speciation of the metals in the soil with the use of X-ray absorption spectroscopy including synchrotron X-ray fluorescence microprobe mapping, microbeam X-ray absorption spectroscopy, and bulk sample X-ray absorption spectroscopy.

Bioaccumulation and Toxicity Models

- The predictive ability of a number of different models relating soil properties to oligochaete metal bioaccumulation as a screening tool for estimating metal bioavailability in soils was examined.
- Key elements for predicting bioaccumulation of metals by soil invertebrates include total metal concentration in the soil, soil physicochemical characteristics, and time.

Plant Bioaccumulation

- Metal phytoavailability was estimated from soil-property driven multiple regression models developed using bioaccumulation data from two previous studies as well as soil extraction methods.
- Comparison of the actual contaminant phytoaccumulation from bioassays with predicted toxicity from *in vitro* models.

In Vitro Testing

- Determine the ability of *in vitro* gastrointestinal methods (i.e., bioaccessibility methods) to predict measured contaminant bioavailability in contaminated soils from study sites.

Metal Speciation Results: Findings from synchrotron X-ray studies indicated that Pb is adsorbed as divalent ions or present as organic complexes, rather than in crystalline compounds. Cr and As are present in their more stable and less toxic inorganic forms, Cr(III) and As(V), except in soil from the Naval Complex at Pearl Harbor, where both As(III) and As(V) are present. Arsenic is bound to iron oxides in the Concord and Pearl samples, and to aluminum oxides in the Hilo soil sample. Arsenic-bearing soils may require more site-specific approaches to remediation. Lead was not bound in sulfide phases that would be considered stable, meaning that most of the Pb-O in the soils may be liberated under acidic conditions (i.e., in the stomach).

Bioaccumulation and Toxicity Models Results: When applying literature-based metal bioaccumulation models to assess Cd and Pb bioaccumulation by earthworms in metal-contaminated field soils, 98% of the variability in earthworm Cd concentrations could be predicted by a model comprising total soil Cd, organic matter content, and soil pH, while 95% of the variability in earthworm Pb concentrations could be predicted by a model including total soil Pb and soil pH. However, both these models over-predicted metal bioaccumulation (Cd Root Mean Square Error [RMSE] - 106%; Pb RMSE - 272%) so their use in predicting bioaccumulation may be limited. A large portion of the variability in the tissue concentrations of As (R^2 - 90%), Cr (R^2 - 77%), and Nickel (Ni (R^2 - 88%) could be estimated by their concentrations in soil. Even though just a few bioaccumulation models exist for these metals, the models for As (RMSE - 24.2%) and Cr (RMSE - 13.6%) provided acceptable predictions of metal uptake, while the Ni model severely over-predicted uptake (RMSE - 689%). However, for the essential metals copper (Cu) and zinc (Zn), total soil concentrations combined with soil properties provided a reasonable prediction of tissue concentrations for Cu (RMSE - 24.7%) but not for Zn (RMSE - 590%). A model relating bioaccumulation factor (BAF) of Cd to soil properties provided acceptable predictions of Cd BAFs by *En. crypticus* from Environmental Security Technology Certification Program (ESTCP) soils (RMSE - 20%) while no relationship was evident between BAFs and observed metal burdens for Pb and Zn.

Models developed relating 0.5 molar (M) calcium nitrate ($Ca(NO_3)_2$)-extractable Cd and Pb to earthworm metal residues did not provide a better prediction of Cd and Pb concentrations in earthworms exposed to ESTCP soils than models selected from the literature that predicted earthworm metal concentrations based upon total metal levels and soil physicochemical characteristics. Models incorporating toxicokinetics of metals were only available for Cd and provided reasonable estimates of Cd concentrations in earthworms (RMSE - 19%). These results

indicate that there are no models for a specific metal that would provide good predictions of metal bioaccumulation in all soils and situations.

Plant Bioaccumulation: The predictive capability required by a soil property/soil extraction model depends on the degree of accuracy of contaminant phytoaccumulation determined by the risk assessor. With some exceptions, both methods were able to predict phytoavailability at RMSE<35% of the measured contaminant tissue value. In general, soil property models were predictive of tissue As, Cd, and Pb. Exceptions were Deseret for As (ryegrass), Hill for Cd (lettuce), and Portsmouth for Pb. In general, the predictive capability of soil extraction methods was adequate to excellent with the exception of Hill for Cd (lettuce) and Portsmouth for Pb.

In Vitro Testing: Equations used to predict bioavailability from bioaccessibility methods are available for Pb and As. Relative bioavailable Pb was determined for the Portsmouth soil in our study. The Physiologically Based Extraction Test (PBET) methods (pH 1.5 and 2.5) were able to accurately predict *in vivo* relative bioavailability (RBA) for the Portsmouth soil. The predicted RBA for the PBET method at pH 2.5 was closer to actual *in vivo* RBA than pH 1.5. However both methods predict RBA Pb within the 90% confidence interval. The Ohio State University *In vitro* Gastrointestinal Method (OSU IVG) method *in vitro* bioaccessible (IVBA) Pb was very close to the *in vivo* RBA Pb. However, information on the ability of the OSU IVG method to predict RBA Pb is very limited whereas in depth validation studies have been conducted for the relative bioaccessibility leaching procedure (relative bioaccessibility leaching procedure [RBALP] i.e., PBET) method. These results support the use of the PBET method at pH 1.5 and 2.5 to accurately predict *in vivo* RBA Pb. Future validation studies where this approach is expanded from the Portsmouth soil to other DoD soils will increase the confidence of using *in vitro* methods to predict *in vivo* RBA Pb.

Results from our study show both the OSU IVG and Solubility Bioavailability Research Consortium (SBRC) method were able to predict RBA As in the Deseret soil. The predicted RBA As by all methods ranged from 12.2% to 16.2%, which is comparable to the *in vivo* RBA As of 14%. Further validation studies of these methods for other contaminated soils from different DoD contaminant sources are warranted. A study investigating the relationship between IVBA Cr and *in vivo* RBA Cr has not been reported. Thus, it was not possible to evaluate the ability of bioaccessible Cr to predict *in vivo* RBA Cr. In our study, a novel immature swine dosing model was used to determine the *in vivo* RBA Cr for the McClellan soil. RBA Cr was 107% with a 90% confidence interval ranging from 76% to 169%. IVBA Cr PBET method, used to measure bioaccessible Cr at pH 1.5 and at pH 2.5, was 10.1% and 19.0%, respectively. The IVBA values were much lower than the *in vivo* RBA Cr. Further research is needed before IVBA can be used to predict *in vivo* RBA Cr.

In general, all of the *in vitro* methods predicted *in vivo* RBA As with 90% confidence.

The ability of soil properties to predict As and Cr bioaccessibility (IVBA) was dependent on the contamination source. In general, IVBA As measured by PBET and OSU IVG could be predicted from measured soil iron (Fe) properties including iron reactive oxide fraction (Feox) or citrate-bicarbonate-dithionite extractable iron (CBD Fe) for soils where arsenical pesticide was the contaminant source. However, properties of the Deseret soil, where mine tailing was the

contaminant source, was not predictive of the measured IVBA As. This finding suggests arsenic may occur as discrete minerals from the mining operation. It is likely the insoluble As minerals in the mining waste did not appreciably dissolve and react with soil components. Therefore, its chemical speciation and IVBA solubility will depend on the mining waste mineral not soil property.

The ability of soil properties (i.e., clay, organic and inorganic carbon [C]) to predict and Cr bioaccessibility (IVBA) was dependent on the contamination source. Good agreement between the measured IVBA Cr and predicted IVBA Cr was found for Hill and McClellan soils. Poor agreement between the measured IVBA Cr and IVBA Cr predicted by soil properties was found for the Cherry Point soil. Differences in Cr chemical speciation in soil may offer an explanation. Water or wastewater treatment was the contaminant source for the Hill and McClellan soils. Incinerator ash was the contaminant source for the Cherry Point soil.

Summary of Soil Properties to Predict Metal Bioavailability

Soil properties, able to predict metal (bio) availability for several contaminated soils in this study, are summarized in Table 11. At a minimum, soil property information needed from a site investigation for all contaminants studied are soil pH, clay content, organic C, inorganic C, reactive Fe and aluminum (Al) (amorphous iron and aluminum oxides [FEAL], Feox and/or CBD Fe). Other properties not studied that will affect ecological endpoints include soil salinity and the presence of other toxicants.

These properties will **not** predict metal bioavailability for all soils. A major finding of this study is the contaminant source and likely speciation greatly affects the ability of soil property to predict metal bioavailability. Metal bioavailability was not able to be predicted for several soils where the contaminant source was unweathered mining waste or discrete inorganic mineral forms such as coal ash. Soil properties should NOT be used to predict contaminant bioavailability in these soils. More research on contaminant source and speciation is needed to determine when soil properties can provide an accurate assessment of metal bioavailability. Currently research is in progress, including research funded by SERDP (i.e., ER-1742) to determine the relationship between As speciation and ability to predict As bioavailability to humans.

Summary of Soil Extraction Methods to Predict Metal Bioavailability

Soil exaction methods, able to predict metal (bio)availability for several contaminated soils in this study, are summarized in Table 12. Both PBET and OSU IVG were able to very accurately predict RBA As and Pb but for only for 1 soil each. The number of soils evaluated were very limited because of cost constraints associated with in vivo dosing trails required to measure contaminant RBA. More research is needed to evaluate the ability of these methods to predict RBA Pb and RBA As on other contaminated soils.

Soil pore water was able to predict plant tissue concentration of Pb, As, and Cd. Soil extraction with 0.1 M $Ca(NO_3)_2$ was able to predict cationic metal contaminants (i.e. Pb, Cd) but was not evaluated for anionic As contamination. The ability of simply water or dilute calcium nitrate to predict phytoavailable contaminant suggests high solubility of these contaminants in soils. Thus,

it is likely that with 0.1 M $Ca(NO_3)_2$ would have also been a good predictor of plant As. However, two cautions should be heeded. The accuracy of these extraction methods to predict plant tissue contamination was limited to ± 35%. Similarly to metal bioaccessibility results, metal bioavailability was not able to be predicted for several soils where the contaminant source was unweathered mining waste (i.e. Deseret) or discrete inorganic mineral forms such as coal ash (i.e. Cherry Point). Soil extraction methods listed in the Table 12 should NOT be used to predict contaminant bioavailability in these soils. More research on contaminant source and speciation is needed to determine which soil extraction methods can provide an accurate assessment of metal bioavailability.

IMPLEMENTATION ISSUES

As part of Objective 3, most of the technical objectives, methods, results, discussion, conclusions, and recommendations of this study are detailed in Appendices A-F of the Final Report, which were written as stand-alone manuscripts for submission as peer-reviewed publications. Publication in peer-reviewed journals is needed to disseminate and ultimately facilitate the results of this study to site managers. In addition, publication in peer-reviewed literature is crucial to ensuring regulatory and community understanding and acceptance of the scientific results. The publication of the results of this study are proceeding.

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1.0 INTRODUCTION

1.1 BACKGROUND

There are thousands of metal-contaminated sites on U.S. Department of Defense (DoD) lands awaiting remediation and closure. Lead (Pb), arsenic (As), chromium (Cr), and cadmium (Cd) are toxic (i.e., capable of producing an unwanted, deleterious effect on an organism) metals of particular concern since these metals often control risk-based remedial decisions for soils at DoD sites [1]. Ingestion of contaminated soil by children is the exposure pathway that generally controls remediation goals [2, 3]. With the exception of Pb-contaminated soils, the risk posed by soil ingestion is currently calculated from the total metal (e.g., as measured by U.S. Environmental Protection Agency [EPA] Method 3050B [4]) concentration and the allowed reference dose (non-carcinogen) or cancer slope factor (carcinogen). Reference doses and cancer slope factors are available for most metals and are typically derived from studies of very soluble metal species. In other words, with the exception of Pb, EPA's risk assessment guidance implicitly assumes a default relative bioavailability of 100%. For the purposes of this study, "bioavailability" refers to the *in vivo* availability of a contaminant to a biological organism (e.g., a plant, human child, or earthworm), while "bioaccessibility" refers to the amount of a contaminant that can be extracted in an *in vitro* procedure. Ruby et al. [5] provides precise definitions of these and other relevant terms (e.g., relative versus absolute bioavailability, etc.). The toxicity assessment for Pb is unique and is based on a pharmacokinetic model of blood Pb. The default bioavailability assumptions in EPA's blood-Pb model are 50% for food and water and 30% for soil, thus yielding a relative bioavailability in soil of 60% (30%/50%).

Metals in soil, however, can be relatively insoluble and sometimes require aggressive digestion procedures for complete analytical metal recovery. As a result, reference doses developed from studies using soluble metal species may overstate the risk posed by less soluble metals in soils. The generally low bioavailability of Pb and As in mining areas has been well documented. Numerous studies, for example, have shown that Pb in soil [6, 7], mining waste [8, 9] and aggregate [10, 11] is much less bioavailable than more soluble Pb species such as Pb oxide, nitrate, or acetate commonly used in toxicological studies. As a result, Pb in mining environments often exhibits limited bioavailability, and children in Pb mining communities often have lower blood Pb levels than in other areas of the country [12]. Relatively low Pb bioavailability is a consequence of Pb speciation and the corresponding solubility constraints [13] and of kinetically-controlled dissolution due to limited residence times in the gastrointestinal (GI) tract [14]. Risk assessments based on data from studies using soluble metal salts overestimate the risk posed by these soils [15]. In mining-impacted areas, low soil-metal bioavailability is most likely due to the presence of residual low-solubility metal.

Recent Strategic Environmental Research and Development Program (SERDP) research on certain U.S. Department of Energy (DOE) and DoD hazardous waste and firing range contaminated soils found that nearly all soil-bound Pb was bioaccessible (measured as an *in vitro* surrogate for oral bioavailability). These data were in agreement with highly labile Pb in Pb-spiked soils from around the country that suggested Pb bioaccessibility remained high despite the fact that it was thoroughly adsorbed to various mineral constituents in the soils [16]. Molecular speciation analyses using x-ray absorption spectroscopy (XAS) suggested that Pb(II) was weakly associated with the soil via electrostatic interactions. Apparently in these systems, weak surface

bonds between Pb and soil are easily disrupted by the acidic conditions encountered in the stomach. This makes Pb much more bioavailable relative to Pb in mining soils where it most likely exists as sparingly soluble lead sulfides (PbS). However, not all DoD soils have highly bioaccessible Pb, as molecular speciation (e.g., metallic or precipitated as sparingly soluble species) can significantly reduce Pb bioaccessibility (Fendorf, Stanford University, unpublished data).

The reference dose for As is based on human epidemiological studies of As in drinking water. However, soluble As in drinking water is much more bioavailable than insoluble As in soils, the latter being primarily excreted through the feces without absorption in the GI tract [17]. Estimates of risk due to ingestion of As-contaminated soils from some areas will be overstated unless the lower bioavailability of As in these soils is considered [18]. Rodriguez et al. [19] found that the *in vivo* relative bioavailability of As in soils from various mining and smelter sites ranged from 3 to 43%. They further found that a physiologically-based *in vitro* bioaccessibility method correlated extremely well with the *in vivo* method that used immature swine as a model for the gastrointestinal function of children.

Recent SERDP research has also shown that reference dose criteria used for soil As and Cr is often highly conservative because the indigenous metal-sequestering properties of many soils can significantly lower the bioavailability of ingested toxic metals relative to commonly used default values [16, 20-22]. Our previous results, for example, have shown that numerous DoD soils throughout the U.S. can effectively sequester As(III/V) and Cr(III/VI), significantly decreasing metal bioavailability. Certain soil physical and chemical properties (e.g., iron [Fe]-oxide content, organic matter content, and pH) were highly correlated with decreased metal bioaccessibility, and statistical models were formulated to estimate metal bioaccessibility. We also used high-resolution spectroscopic techniques, such as XAS, to characterize the chemical environment and speciation of sequestered metals and to verify the modeling results. Studies conducted at DOE's Stanford Synchrotron Radiation Laboratory (SSRL) confirmed that numerous DoD soils contain natural soil constituents that could reduce mobile Cr(VI) to the less toxic Cr(III) species and oxidize highly mobile As(III) to the less mobile As(V) species. These redox transformations significantly decreased toxic metal bioaccessibility. Nevertheless, certain soil conditions were also found to enhance bioavailability of these metals. For example, when the soil Fe-oxide content for a particular DoD soil fell below 0.5% on a mass basis, the bioaccessibility of As increased dramatically, particularly for alkaline soils [16, 20]. Likewise, for DoD soils low in organic and inorganic carbon, the bioaccessibility of Cr(III) and Cr(VI) is significantly higher relative to soils that possessed these mineral constituents [21, 22].

Unlike Pb and As, most studies of zinc (Zn), copper (Cu), Cd, and nickel (Ni) bioavailability in soils have focused on ecological bioavailability, primarily plant uptake. It is unlikely that a soil extraction method will replicate the amount of metal absorbed by plants. The plant uptake system is too complex and dynamic to simulate by simple extraction methods in the laboratory. A more reasonable approach may be to use soil extraction methods that are based upon soil chemistry and root physiology and that correlate well with plant uptake of metals. The discipline of Soil Science has used this very concept successfully for the last 50+ years. Chemical extractants cannot extract plant nutrients in the same manner as a living plant under the conditions of the plant root environment. However, good correlations between soil extracts and

plant uptake has allowed soil scientists to use that relationship to make reasonable predictions of plant available nutrients in soil and subsequent fertilizer recommendations. Plant uptake studies have shown that these metals are largely immobilized by soils, and only a small fraction is bioavailable. Banjoko et al. [3] found that most of the zinc (78%) present in soil existed in the recalcitrant residual fraction and was not available to maize grown in the soils. When Zn was added to the soil, the calcium (Ca)-exchangeable fraction decreased to zero within a few days, reflecting the increasing strength of the metal-soil bond over time. Pierzynski [23] found that uptake of Zn by soybeans correlated not with total soil Zn but with more readily available fractions. Similarly, only a readily available fraction of Cu, Cd, and Ni [24-27] is typically bioavailable in soils. In addition, when metal-scavenging manganese (Mn) [28] or Fe [29] oxyhydroxides are added to soil, metal bioavailability decreases.

Recent SERDP research in our group, using a physiologically-based *in vitro* bioaccessibility method to simulate the human GI tract, has shown that DoD soil-bound metals such as Pb and Cd sometimes remain highly bioaccessible even though they are sequestered by the soil solid phase. Although these toxic metals were effectively bound to the surfaces of mineral constituents in the soil, their weak surface bonds were easily disrupted by the acidic conditions encountered in the simulated stomach environment, allowing them to be much more bioaccessible. These findings are consistent with several bioavailability studies documented by the National Environmental Policy Institute [30] that confirm soils decrease the bioaccessibility of Cd but not nearly to the extent as is observed for metals such as As and Cr. Schroder et al. [31] reported a mean bioaccessible Cd of 63.0% using an *in vitro* gastrointestinal method and mean Cd relative bioavailability of 63.4% in contaminated soils from dosing trials using immature swine. Based on these findings, measurements of key soil properties could be used as indicators to determine whether site remediation is necessary or if more definitive site-specific *in vivo* metal bioavailability studies are warranted. However, site-specific use of bioavailability estimates from soil properties is impeded by the lack of regulatory acceptance. This is rational due to the lack of site-specific investigations that couple *in vivo* bioavailability and *in vitro* bioaccessibility studies with soil properties and microscopic interrogation of the solid phase metals. Several studies have shown good correlations between the *in vitro* Physiologically Based Extraction Test (PBET) or *In vitro* Gastrointestinal (IVG) methods and *in vivo* swine feeding studies for soil Pb [32], soil As [19], and soil Cd [31]. However, none were specifically designed to investigate DoD site-specific soils or considered the role of soil properties in controlling metal bioavailability.

On DoD sites where human exposure is not the main cleanup driver and an ecological risk assessment (ERA) is required, metal bioavailability must be estimated by methods other than PBET or IVG extractions in order to assess exposure for wildlife, soil invertebrates, and plants. Although these extraction techniques may serve to estimate dietary metal exposure in mammalian wildlife, they would not suffice for exposure estimates for soil invertebrates and plants. Similar to human exposure estimates, bioavailability is not currently considered in ERAs and exposure dose is measured as total metal levels. Instead of references doses, toxicity reference values (TRV) and ecological soil screening levels (EcoSSLs) have been developed by EPA for screening soil metal levels for wildlife, soil invertebrates, and plants. These values have been developed considering soils in which metals are maximally bioavailable. However, site-specific bioavailability adjustments are possible if site metal levels are found to exceed these screening values. A number of techniques are available for making bioavailability adjustments

for metals exposure to soil invertebrates and plants. Weak salt extractions (e.g., calcium nitrate [$Ca(NO_3)_2$] or calcium chloride [$CaCl_2$]) offer a reasonable alternative to total metal levels and are currently being employed as an additional method for estimating the bioaccessible fraction of metals in soils.

1.2 OBJECTIVE OF THE DEMONSTRATION

The technical objectives of the investigation are:

1. To provide validation that the relationships between soil properties and *in vitro* bioaccessibility methods can serve as a screening tool for estimating *in vivo* toxic metal bioavailability in DoD soils;
2. To provide DoD with a scientifically and technically sound method for estimating human and ecological risk associated with metal contaminated soils in place of or as justification for more-detailed, site-specific bioavailability (e.g., animal dosing); and
3. To promote the use of *in vitro* methods in human health and ecological risk assessments through the upfront involvement of end-users and regulators and the subsequent dissemination of the results of the study in peer-reviewed journals.

1.3 REGULATORY DRIVERS

Several recently published studies have summarized the current regulatory climate in regards to these issues. For example, Ehlers and Luthy [33] summarized the results of the recent National Research Council (NRC) report "Bioavailability of Contaminants in Soils and Sediments." There is neither a national policy nor legal recognition of incorporating bioavailability considerations in site cleanup, although individual states have allowed bioavailability adjustments on a case-by-case basis [5]. To help fill this void, EPA is developing guidance and hosted an expert panel discussion in April 2003 on metal bioavailability in soils. Several factors must be aligned at a site to make bioavailability of a contaminant an important consideration: 1) the contaminant whose bioavailability is being investigated is the risk driver; 2) default assumptions of 100% bioavailability are unrealistic; and 3) substantial quantities of contaminated soil and sediment are involved. Bioavailability arguments should also only be used where site conditions (e.g., land usage, biogeochemical environment, etc.) are unlikely to change over the relevant timeframe. The report advocates long-term monitoring of contaminant sequestration. A range of tools is available to study bioavailability, from microscopy, to chemical extractions, to bioassays. Tools that promote mechanistic understanding and lead to the development of a predictive capability are preferred over empirical approaches. Although the report provides a nice ranking of tools, no single tool achieves the highest ranking in all categories. The report thus advocates a "weight-of-evidence" approach to tool selection. The default assumption is typically 100% contaminant bioavailability, which is usually a conservative assumption, because most toxicity tests intentionally use forms of chemicals that are readily absorbed. Bioavailability assessments can be used to help better prioritize site cleanup. Most previous assessments have usually come from industry-funded studies at specific sites.

Studies have also focused on the application of these techniques specifically to DoD sites [34, 35]. Except for Pb, the EPA's human health risk assessment guidance implicitly assumes a

default relative bioavailability of 100%. Bioavailability data can be incorporated into risk assessments at the screening level (Tier IB) as well as in the baseline risk assessment (Tier II). The results of the Tier IB assessment can be used to remove sites from further consideration or for early identification as to whether or not a bioavailability adjustment is potentially useful in the baseline risk assessment. Bioavailability adjustments should be considered in the following situations: a) a risk estimate slightly or moderately exceeds an acceptable level and triggers required remediation; b) risk-based cleanup goals require extensive remediation; c) remediation is not technically feasible; and d) remediation will adversely impact the environment. If more than three chemicals are risk drivers at a given site, the chances that bioavailability adjustments of a few would significantly affect the required cleanup levels are lessened. Factors that significantly affect whether or not a bioavailability study should be considered include: a) whether the studies can be completed within the required timeframe; b) the cost of the bioavailability study relative to cleanup; c) whether or not existing data support the likelihood of reduced bioavailability.

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2.0 TECHNOLOGY

2.1 TECHNOLOGY DESCRIPTION

The purpose of this demonstration was to demonstrate the ability of soil chemical and bioassay methods to predict metal bioavailability for human and ERA. The project sought to provide validated evidence that *in vitro* bioaccessibility methods can serve as time- and cost-effective predictive indices of toxic metal bioavailability in DoD soils relative to *in vivo* uptake studies. By quantifying the extent to which soil properties control metal bioavailability, we have shown that the models developed in our previous SERDP projects can be used with reasonable confidence to predict site-specific metal bioavailability for DoD soils throughout the United States. By coupling *in vitro* and *in vivo* methods at numerous DoD field scale facilities with upfront regulator and end user input, our goal is to facilitate regulatory acceptance of *in vitro* methods and predictive tools for assessing toxic metal bioavailability in contaminated DoD soils as it relates to human and ecological risk.

Soil properties, total metal content, speciation, and bioaccessibility and bioavailability (as measured by various *in vitro* and *in vivo* methods, respectively) were determined for metal contaminated soils collected from three DoD sites for the human health models. A similar approach was taken for the *in vitro* ecological model, which was made more robust by considering an additional eight DoD soils (total of eleven contaminated and eleven control soils for the ecological models).

Human Health: Metal bioaccessibility and metal bioavailability for three study soils was calculated using soil property-driven models developed from our earlier SERDP studies. Calculated bioaccessibility values were compared with measured bioaccessibility values using *in vitro* GI methods for study soils. The PBET developed by Ruby et al. [5], was utilized at a variety of pH conditions to estimate metal bioaccessibility for a variety of stomach environments indicative of food intake, or lack thereof. Using the method of Stewart et al. [21, 22], additional soil property-driven models were constructed using the PBET method at these pH values. This is particularly important for Pb contaminated soils since Pb bioaccessibility decreases with an increase in pH [20, 36]. In contrast, As(V) bioaccessibility was minimally influenced by changing pH environments. In addition to PBET, the Ohio State University *In vitro* Gastrointestinal Method (OSU-IVG) [37] method was used to measure bioaccessible As. The ability of the OSU-IVG method to predict contaminant bioavailability was determined.

Ecological: For ecological risk estimates, metal bioavailability was estimated from multiple regression models developed using bioaccumulation data from 26 soils from the EPA- National Center for Environmental Assessment (NCEA) study [38-43]. Also, the ability of soil extraction methods to predict phytoavailable metals were investigated. Additionally, eight selected DoD sites were tested in addition to the three soils used in the swine study. This was necessary to enhance the robustness of the ecological model [38-43] as has already been done for the human-based model in ER-1166. In the ecological investigations, metal concentrations from *in vitro* DoD soil metal extractions or DoD soil chemical and physical properties were used to predict metal bioavailability to plants and soil invertebrates. Initially, statistical relationships developed for metal availability from a set of twenty-six soils were used to estimate the chemical availability of metals in DoD soils, based upon total metal levels and soil physical/chemical

characteristics. This was followed by extraction of the DoD soils using several soil extraction methods using pore water, dilute calcium nitrate solution, and Mehlich 3 solution. The ability of soil chemical extractants to predict metal bioavailability to plants was determined. Plant and soil invertebrate bioassays were conducted with DoD soils to determine actual toxicity and bioaccumulation, and these results were compared to the model predictions of toxicity and bioaccumulation.

2.2 TECHNOLOGY DEVELOPMENT

Human Health: Within SERDP ER-1166, a predictive model, the Soil BioAccessibility Tool (SBAT) [44] was developed to assess the relative bioavailability of toxic metals in soils. The model was built on the premise that key soil physical and chemical properties (e.g., Fe-oxide content, organic matter content, pH) were statistically correlated with metal bioaccessibility (as measured by *in vitro*, PBET technique). Model results were found to be in good agreement with molecular level metal speciation studies and *in vivo* swine feeding studies [20, 36]. Nevertheless, model validation using *in vivo* studies on actual DoD field samples was lacking. Such an endeavor is critical if the model is ever to obtain end-user and regulatory acceptance.

In addition, recent publications within our group, investigating the bioavailability of As in soil have found that an *in vitro* bioaccessibility method correlated extremely well with the *in vivo* method that used non-DoD soils and immature swine as a model for the GI function of children [19]. Similar findings have been reported for soil bound Pb and Cd where the *in vitro* PBET method correlated very well with *in vivo* swine feeding studies [31, 32]. The OSU-IVG method has been shown to be correlated with As [37], Pb [45], and Cd [31]. Our research team members also belong to the Bioavailability Research Group of Europe (BARGE) where we have established an international collaboration that seeks to demonstrate the appropriateness of *in vitro* methods for assessing risk associated with soil metal bioavailability. The United Kingdom and several countries within the European Union have used our (United States) data of coupled *in vitro* and *in vivo* soil metal bioavailability to convince the regulatory community, in their respective countries, that *in vitro* measurements of soil metal bioaccessibility are acceptable estimates of *in vivo* soil metal bioavailability, at least at mining sites. However, although site-specific bioavailability adjustments have been made at some sites, regulators in the United States remain uncertain that the *in vitro* methods alone can adequately predict soil metal bioavailability in humans.

Ecological: Prior ecotoxicological studies within our group have also been completed that show soil properties similarly affect the bioavailability of As, Cd, Pb, and Zn for soil invertebrates and plants. Measures of metal exposure based upon soil extraction techniques, such as dilute salts [42, 43, 46, 47], have been coupled with soil chemical and physical properties to develop statistical relationships for estimating metal bioavailability for soil organisms. These statistical models are the first step in the development of models capable of predicting the toxicity of metals to soil invertebrates and plants.

Based on our previous scientific and technical advances in the area of *in vitro* and *in vivo* metal bioavailability in soils, we believed that it was timely to apply these techniques to DoD site-specific problems. Such an effort would validate bioaccessibility and bioavailability estimates based on *in vitro* methods and soil properties for DoD sites. Close cooperation with regulators

and end users would lead us closer to regulatory acceptance of *in vitro* methods for assessing toxic metal bioavailability in soils and use of the validated predictive tool SBAT.

Our team has also been involved in research addressing the ecological risk of metals in soil systems. Basta, Dayton and Lanno conducted soil ecotoxicological research for a EPA-NCEA research project "An Integrated Soil Chemical and Toxicological Approach for the Development of Ecological Screening Levels for Heavy Metals in Soil" (NCEA-ORD Award # CR 827230-01-0) that involved developing methods for determining metal exposure in soil to earthworms and plants using chemical analysis methods other than total metals. Experiments were conducted in twenty-two soils differing in physical/chemical characteristics to develop statistical models relating soil characteristics to bioavailable levels of metals and toxicity in plants and earthworms. The results of our research have also lead to studies examining the physiological partitioning of metals in soil invertebrates and collaborations with researchers at Rijksinstituut voor Volksgezondheid en Milieu (RIVM) (Bilthoven, The Netherlands) and the Vrije Univeristeit (Amsterdam, The Netherlands).

2.3 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

The goal of this initiative was to provide field-validated evidence that *in vitro* bioaccessibility methods can serve as predictive indices of toxic metal bioavailability in DoD soils relative to the more costly and time intensive *in vivo* uptake studies. By quantifying the extent that soil properties control metal bioavailability, we have shown that the predictive models developed in our earlier SERDP studies can be used with a reasonable level of confidence to predict site-specific metal bioavailability for DoD soils throughout the United States. We believe that this upfront investment by Environmental Security Technology Certification Program (ESTCP) to compare *in vitro* methods with *in vivo* investigations can potentially save DoD significant remedial cost in the long term.

The lack of wide-spread regulatory acceptance of the *in vitro* methods is the largest potential limitation to widespread application. Another potential limitation with using this technology at DoD sites is that there are different types of metal-contaminated sites within the DoD, e.g., small arms firing ranges, paint residues, past pesticide use, and manufacturing/maintenance activities. The bioavailability of a given metal could vary widely between sites, underscoring the ultimate need for site-specific adjustments.

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3.0 PERFORMANCE OBJECTIVES

One of the performance objectives was to test the bioavailability screening tools developed in our earlier SERDP studies, which correlate chemical speciation, bioaccessibility, bioavailability, and toxicity of metals (Pb, As, Cd, Cr) in DoD soils as measured by biological models used to evaluate ecological risk (e.g., plants, earthworms) and human risk (e.g., immature swine model) (Table 1). Since ingestion is often the primary human risk driver at contaminated sites [1], human risk by ingestion was evaluated rather than dermal pathways. Only three sites were considered for the *in vivo* swine dosing studies due to the experimental cost. The use of *in vitro* ecological models were further verified by comparison with *in vivo* ecological bioassay studies of eleven DoD soils (eleven contaminated, eleven control). At the kickoff workshop, the research strategy was discussed among scientists, regulators, EPA, and end-users to advance the acceptance of *in vitro* methods in human health and ERA and policy.

Table 1. Performance objectives.

Performance Objective	Data Requirements	Success Criteria	Results
Quantitative Performance Objectives			
Ecological bioassays versus *in vitro* protocol	Agreement between the measured and empirical model-predicted bioavailability	Significant multiple regression correlation criteria and/or Root Mean Square Error (RMSE) ≤25%	Soil Inverterbrate-Yes[1] Plants-Yes
	Toxicity and bioaccumulation consistent with speciation	Predictive ability of model confirmed	Soil invertebrates – Mixed[2] Plants-Yes
	Estimated risk	Bioassay Hazard Quotients (HQ) and *in vitro* HQs	Soil Inverterbrate-Mixed[3] Plants-Yes
Swine bioassays versus *in vitro* protocol	Agreement between the measured and empirical model-predicted bioavailability	RMSE ≤25%	Pb and As-Yes Cr-No
	Toxicity and bioaccumulation consistent with speciation	Predictive ability of model confirmed	Pb and As-Yes Cr-No
Qualitative Performance Objectives			
Technology transfer	End-user involvement	Kick-off meeting held and comments of end-users incorporated in research design.	Yes
Ecological bioavailability protocol	Protocol is applicable for evaluating Pb, Cd, Cr, As in soil	Validated statistical model	Soil invertebrates – Mixed Plants-Yes
	End-user acceptance	Results published in peer-reviewed journals.	Pending
Human bioavailability protocol	Protocol is applicable for evaluating Pb, Cr, As in soil	Validated statistical model	Pb and As-Yes Cr-No
	End-user acceptance	Results published in peer-reviewed journals.	Pending

1. Many significant multiple regressions, some acceptable RMSE, not applicable to essential elements, Cu and Zn, that were not at toxic levels and are regulated by the organisms.
2. Speciation did not significantly increase the predictive capacity of bioaccumulation models.
3. Bioaccumulation of metals only, so no HQs; comparison to EPA EcoSSLs did not reveal trends.

An important component of the technical approach is to validate and demonstrate the ability of soil property models [20-22, 36] and *in vitro* techniques to predict metal bioavailability and risk (i.e., ecological, human). Results obtained from methods developed for assessing metal risk-based endpoints for humans in our earlier SERDP studies were compared with results from well-established standard methods used to determine human risk (USEPA Risk Assessment Guidance for Superfund) and ecological risk (USEPA Ecological Risk Assessment).

The agreement between the measured and the model-predicted bioavailability was quantified with the RMSE

$$\mathrm{RMSE} = \left[\frac{1}{n_d - n_p} \sum_{i=1}^{n_d} \left(B_i - \hat{B}_i \right)^2 \right]^{1/2}$$

Where n_d is the numbers of data points, n_p is the number of adjustable parameters (zero when used in a purely predictive manner as in this project), i is an index, and B_i and $\hat{B}_i$ are the *i*-th measured and predicted bioavailability, respectively. The RMSE, the square root of the mean squared difference between measured and predicted values, is a measure of the average error between the predicted and measured values. Our goal was for our models to produce RMSE ≤ 25%.

Overall performance objectives are shown in Table 1. A discussion of these performance objectives as well as supporting performance objectives can be found in Appendices A-F of the Final Report.

4.0 SITE DESCRIPTION

4.1 SITE LOCATION AND HISTORY

The following three sites were selected for the swine dosing studies:

- Portsmouth Naval Shipyard
- McClellan Air Force Base (AFB)
- Deseret Chemical Depot

The following sites were used for the ecological bioavailability and *in vitro* bioaccessibility studies:

- Hill AFB
- Travis AFB
- Marine Corp Air Station (MCAS) Cherry Point
- Naval Support Activity (NSA) Mechanicsburg
- Portsmouth Naval Shipyard
- McClellan AFB
- Deseret Chemical Depot
- Concord Naval Weapons Site
- Former Sugarcane Fields
- Naval Complex, Pearl Harbor, HI
- Oak Ridge National Laboratory (ORNL)

Hill AFB: Hill AFB is located in Ogden, UT. The contaminated area was historically used as sludge drying beds during the treatment of water for potable use.

Travis AFB: Travis AFB is located in Fairfield, CA. Soils from a former small arms range that operated from 1957 until 1977 contain elevated concentrations of lead and antimony.

MCAS, Cherry Point: The MCAS is located in Cherry Point, NC. Soils from a former incinerator site contain elevated concentrations of chromium.

NSA, Mechanicsburg: The NSA is located in Mechanicsburg, PA. Soil from Site 11, which has functioned as a lead ingot stockpile location from the early 1950s until recent years, is heavily contaminated with lead.

Portsmouth Naval Shipyard: The Portsmouth Naval Shipyard is located in Kittery, ME. Soils from Site 6 are impacted by particulate deposition from historical land use as a temporary storage area of a variety of materials, including lead battery cell plates.

McClellan AFB: McClellan AFB is located in Sacramento, CA. Soils from a former wastewater treatment lagoon are contaminated with high concentrations of lead, chromium, and cadmium.

Deseret Chemical Depot: The Deseret Chemical Depot is located in Tooele, UT. Soils from an area that was contaminated with mine tailings from flooding during the 1930s were selected.

Concord Naval Weapons Station: The Concord Naval Weapons Site is located in Concord, CA. Soils from a site that contains elevated As from pesticide applications were utilized.

Former Sugar Cane Fields: Former sugar cane fields located in Hilo on the big island of Hawaii contain high concentrations of As. The use of As-based pesticides during the 1920-1940s is believed to be the source of the contaminant.

Naval Complex, Pearl Harbor: Soils located at the Pearl City Fuel Annex contain high levels of As and Pb. The source of As at this site is thought to be historic pesticide or rodenticide use.

Firing Range, ORNL: Soils located on the small arms firing range contain elevated concentrations of lead.

4.2 SITE GEOLOGY/HYDROGEOLOGY

The soils types and soil physical and chemical properties are shown in Table 2 and 3. Please see Appendices A and F of the Final Report for more detailed soil characterization.

Table 2. Test sites and soil types.

Site Name	Site Location	Soil Type
Travis AFB	Fairfield, CA	Alfisol
McClellan AFB	Sacramento, CA	Alfisol
Hill AFB	Ogden, UT	Entisol
Portsmouth Naval Shipyard	Kittery, ME	Inceptisol
NSA	Mechanicsburg, PA	Ultisol
MCAS Cherry Point	Cherry Point, NC	Entisol
Deseret Chemical Depot	Tooele, UT	Aridisol
Concord Naval Weapons Site	Concord, CA	Vertisol
Naval Complex, Pearl Harbor	Honolulu, HI	Mollisol
Former Sugar Cane fields	Hilo, HI	Andisol
ORNL Firing Range	Oak Ridge, TN	Ultisol

Table 3. Select soil properties of contaminated soil (C) and reference (i.e., uncontaminated) soil (R).

All soils are < 2 mm fraction.

		Cherry Pt		Concord		Deseret		Hill	Hilo		McCllelan	
	Units	C	R	C	R	C	R	C	C	R	C	R
Soil pH, water		5.50	7.43	6.67	6.34	9.28	7.84	7.22	5.88	4.71	4.31	6.66
Soil pH, $CaCl_2$		5.01	6.96	6.15	5.89	7.49	6.91	7.08	5.74	4.73	4.32	6.08
EC	dS/m	0.892	0.353	0.111	0.189	0.544	0.480	0.989	0.820	1.53	0.276	0.119
Alox	mg/kg	6061	909	1522	1672	786	1207	1175	21344	5917	2175	487
Feox	mg/kg	7506	797	3664	4519	863	681	956	25678	7535	4805	804
Mnox	mg/kg	32.2	<25	641	659	313	381	333	484	85.7	<25	125
Org C	%	3.71	0.758	3.13	2.17	0.645	0.792	1.50	7.77	5.69	4.36	0.360
Total C	%	4.54	1.94	3.04	2.13	2.32	1.52	2.66	8.44	5.50	4.66	0.42
CEC	$cmol_c/kg$	9.14	3.94	27.9	27.7	8.37	13.4	11.0	17.1	10.1	13.4	12.0
Sand	%	79.7	80.0	18.4	19.9	36.6	27.5	52.3	61.1	72.3	25.7	59.9
Silt	%	13.5	12.2	40.9	44.3	54.7	53.2	31.3	25.3	17.8	50.2	25.2
Clay	%	6.8	7.8	40.7	35.8	8.7	19.3	16.4	7.8	2.6	24.1	14.9

		Mechanicsburg		ORNL		Pearl City		Portsmouth		Travis	
	Units	C	R	C	R	C	R	C	R	C	R
Soil pH, water		8.04	7.46	4.1	3.81	7.34	7.65	6.2	6.2	7.04	6.02
Soil pH, $CaCl_2$		7.04	7.12	3.53	3.14	7.28	7.47	6.04	5.72	6.46	5.63
EC	dS/m	0.209	0.291	0.184	0.152	0.995	0.929	0.089	0.183	0.247	0.261
Alox	mg/kg	1615	2050	388	851	3502	2046	3764	4149	799	885
Feox	mg/kg	1407	2492	507	798	44900	1977	5758	2682	3088	4569
Mnox	mg/kg	290	944	27.4	<25	1014	492	124	70.1	405	547
Org C	%	0.640	1.22	0.326	0.222	2.34	0.29	1.64	1.44	1.09	1.32
Total C	%	4.49	1.43	0.38	0.17	3.33	2.01	2.57	1.72	1.22	1.39
CEC	$cmol_c/kg$	9.74	9.58	2.79	7.90	25.9	39.4	2.73	2.68	17.3	10.8
Sand	%	29.9	9.90	45.7	9.0	48.7	54.7	89.0	86.5	47.6	29.9
Silt	%	36.6	50.0	36.5	33.4	29.2	26.9	8.5	9.6	26.3	44.3
Clay	%	33.5	40.1	17.8	57.6	22.1	18.4	2.5	3.9	26.1	25.8

Soil pH (water): pH measured in 1:1 soil:deionized water suspension
Soil pH ($CaCl_2$): pH measured in 1:2 soil: 0.01 M $CaCl_2$ suspension
EC: electrical conductivity measured in 1:1 soil:deionized water suspension
CEC: cation exchange capacity
Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction

Table 4. Select properties of ESTCP contaminated soils (C) and reference (uncontaminated) soils (R).

All soils are < 250 μm fraction.

		Cherry Pt		Concord		Deseret		Hill	Hilo		McCllelan	
	Units	C	R	C	R	C	R	C	C	R	C	R
Alox	mg/kg	10897	988	1746	1765	747	1251	1548	28692	none	3415	650
Feox	mg/kg	13216	821	4207	4752	1037	763	1358	30671	none	6248	1482
Mnox	mg/kg	54.3	<25	634	621	293	224	413	635	none	<25	125
Org C	%	5.94	0.97	2.59	1.79	0.48	0.73	2.02	9.42	none	4.56	0.52
Total C	%	7.71	1.62	3.18	2.11	2.00	1.33	3.31	10.6	none	4.42	0.548
CBD Fe	mg/kg	10824	---	12749	---	6044	---	4530	29606	---	6030	---

Alox, Feox, Mnox: reactive oxide fraction measured using acid ammonium oxalate extraction
CBD Fe: citrate-bicarbonate-dithionite extractable Fe

4.3 CONTAMINANT DISTRIBUTION

The contaminant distributions within the soils are shown in Table 5. Please see Appendices A and F of the Final Report for more detailed soil characterization.

Table 5. Metal concentrations in contaminated (C) and reference (R) soils (dry weight basis).

Soil		Cd mg/kg	Pb mg/kg	Cr mg/kg	Ni mg/kg	As mg/kg	Zn mg/kg	Cu mg/kg
Mechanicsburg	**R**	<1.0	33	56	36	17	97	19
	C	<1.0	120	39	29	15	98	25
Cherry Point	**R**	<1.0	17	13	3.5	1.7	32	<1.0
	C	19	114	876	78	6.9	486	167
Travis	**R**	<1.0	17	43	23	8.1	70	19
	C	<1.0	2034	42	29	11	225	148
Concord	**R**	<1.0	16	79	98	7.8	101	50
	C	<1.0	22	77	92	220	112	54
McCllelan	**R**	0.7	15	126	60	6.1	32	14
	C	22	193	699	87	9.9	448	241
Point Loma	**R**	<1.0	8.7	23	6.8	3.7	61	11
Portsmouth	**R**	<1.0	48	14	8.4	10	60	12
	C	1.1	3069	11	62	11	500	185
Deseret	**R**	<1.0	20	27	17	11	83	15
	C	<1.0	19	24	16	438	85	13
ORNL	**R**	<1.0	12	48	15	14	85	14
	C	<1.0	966	16	4.2	5.0	30	65
Pearl	**R**	1.4	13	233	182	4.1	133	110
	C	3.6	1466	185	196	619	1804	423
Hilo	**R**	1.3	153	120	561	22	282	69
	C	5.9	2134	140	417	660	1889	224

Point Loma soil was uncontaminated.

5.0 TEST DESIGN

5.1 CONCEPTUAL EXPERIMENTAL DESIGN

5.1.1 Soil Collection and Characterization

A portable field X-ray fluorimeter was used to identify target metal concentrations in the collection areas prior to collecting 10 to 12 buckets of soil, each containing 25 kg. Since the metal concentration in soil can vary greatly between and within the sample buckets, all soil collected from each site was mixed to produce a homogenous composite sample to be used for all investigations. Although the homogenization procedure described below may have impacted the oxidation state of the target metals, it ensures that the characteristics observed using synchrotron X-ray techniques are the same as those used for *in vitro*, ecological bioaccessibility, and swine-dosing bioavailability tests. The disadvantage is that there may be some differences in soil characteristics compared with the soil in its local environment. These differences are expected to be minimal in that the soil samples were collected from the surface, and therefore already exposed to an oxidizing atmosphere; none of the soils were from wetlands or other reducing environments. The homogenization procedure is not expected to affect distribution of target metals on soil particles, so X-ray fluorescence microprobe mapping provides an accurate record of elemental associations that supports interpretation of the metal distribution on soil particles.

Soils were air dried prior to homogenization in a heavy duty electric powered mixer with a 9 ft^3 plastic drum over six hours. A large cement mixer was modified to allow simultaneous homogenization and sieving (<2 mm) of large amounts (250+ kg) of contaminated soil by using a steel cone attachment fitted with a 2-mm sieve. The steel cone attachment, custom built for the cement mixer, allows (i) greatly improved homogenization, (ii) improved safety by greatly reducing exposure to contaminated dust from the project soils, and (iii) improved efficiency and recovery of homogenized soil. The mixer is equipped with a dust trap to avoid air dispersion of the material. For soils where clumping is an issue, hardened ceramic balls were placed in the mixer with the soil in order to enhance aggregate breakup without grinding the soil, which could alter its native particle size distribution. Soils were next sieved to <2 mm with a subsample sieved to <270 um. The <2mm samples were used in the *in vitro* and *in vivo* plant and earthworm model studies whereas the <270 um samples were used in the *in vitro* and *in vivo* swine model studies and for synchrotron X-ray interrogation. To verify that soil samples are homogeneous, numerous subsamples (10 or more) were acid digested using EPA method 3051a followed by Cr, As, Cd, and Pb analysis. Soils are archived at Ohio State University where *in vitro* and *in vivo* plant and earthworm model investigations were performed.

Select, yet the most pertinent (based on our previous SERDP-funded research), soil chemical and physical properties were quantified using established analytical procedures. The soil properties were measured on all soils are total metal analysis, total organic and inorganic carbon, amorphous and crystalline Fe-oxide content, Mn-oxide content, particle size analysis (sand, silt, clay content), CEC and soil pH. This information was used in the statistical models to assess the influence of soil properties on metal bioavailability as measured by *in vitro* and *in vivo* techniques.

5.1.2 Metal Speciation and Chemical Environment

In an effort to validate the physical significance of the soil property models used to describe the bioaccessibility of metals in the DoD soils, the mechanisms of enhanced metal sequestration and solid-phase metal speciation were quantified with a variety of high-resolution surface spectroscopy techniques. X-ray absorption spectra on bulk samples of the <270 µm size fraction were collected at the SSRL in May 2007 (beam line 2-3; Pb and As analysis) and January 2008 (beam line 11-2, Cr analysis). In both cases a Si(220) monochrometer was used to control the energy of the incident beam, calibrated by metal foils or known reference compounds. Data were collected in fluorescence geometry using a 13- or 30-element germanium solid-state detector (BL 2-3 and BL 11-2, respectively). Samples were ground to fine powder and mounted in teflon sample holders sealed with Kapton tape. Between three and 25 scans were collected on each sample.

Data files were imported into the Samview module of the X-ray absorption spectroscopy processing program Sixpack [48] where monochrometer energy calibration was verified or corrected, and individual scans were examined to ensure that each solid-state detector channel had successfully recorded data. Noise recorded in malfunctioning channels was eliminated before averaging scans. The averaged data was then imported into the program Athena [49]. The near-edge portions of the X-ray absorption near-edge structure (XANES) were examined and first derivatives calculated to determine the energy position of the absorption edge. Next, spectral backgrounds were subtracted and the extended fine-structure portions of the spectra (EXAFS) were expressed in K-space ($Å^{-1}$), where K represents the momentum wave-vector. The resulting χ(K) files were imported into the program Artemis [49] for analysis of the EXAFS.

Least squares fitting algorithms of the EXAFS function were applied to determine nearest and second-nearest neighbor atomic identities, coordination numbers, and distances from the target metal(loid), using theoretical phase and amplitude functions generated by the program FEFF [50]. First-shell coordination environments were identified, informed by the oxidation state information obtained from XANES. The energy offset parameter E_0 was constrained to be the same for all atoms included in the fit. Wave amplitudes corresponding to the coordination number around the target metal were allowed to vary, as were the interatomic distances. The Debye Waller factor, a parameter that varies as a function of static and vibrational atomic disorder [51], was held constant and constrained to be the same for all atoms in the first shell.

For samples containing As, theoretical multiple scattering paths within As tetrahedral were generated from the mineral structure of scorodite ($FeAsO_4 \bullet 2H_2O$). Phase and amplitude functions corresponding to 3-leg paths of the form As-O-O-As (12 paths) and 4-leg paths of the form As-O-As-O-As (16 paths) were generated in Artemis using the IFEFFIT module. To test whether including multiple scattering contributions improved the fit for As K edge EXAFS, the multiple scattering paths were applied with distance and degeneracy parameters fixed to their original values, and the Debye Waller factor constrained to 0.001 [52].

Following first-shell fits, second-shell fits were performed if peaks in Fourier transforms of the EXAFS data representing interatomic distances (uncorrected for phase shift) provided evidence of more distal backscatterers. Potential identities of second-shell backscatterers were informed

by the soil chemical analyses and, when available, results of the X-ray fluorescence microprobe mapping performed at Advanced Photon Source (APS) (described below).

Microbeam X-ray techniques were performed at APS (Argonne National Laboratories) bending magnet beam line 20-BM, operated by the Pacific Northwest Consortium Collaborative Access Team (PNC-CAT), in February 2008. Microbeam X-ray fluorescence (XRF) spectroscopy was used to assess spatial distributions of the target elements on the soil particle surfaces. Soil grains were dispersed onto Kapton tape, covered with a second layer of tape, and placed at a 45E angle to the incident beam. An initial location on the sample with multiple, well-spread out particles was chosen with the aid of a video camera. A constant focal position for all samples was maintained by moving each sample on a motorized rail until it was in focus by a second camera with a viewer outside the hutch. Two-dimensional fluorescence microprobe maps were then acquired to ascertain the distribution of target elements in relation to soil particles.

The images were processed on-site using the PNC-CAT software 2d Scan Plot version 2. Individual element distributions (in relation to the dead-time corrected incident X-ray intensity), and mapped representations of element ratios, were compared visually to detect the areas highest in the target metals to choose locations for collecting microbeam X-ray absorption spectra. In cases where the metal association with other elements was not uniform, more than one spot was chosen. For preparation of X-ray fluorescence map figures, target elements mapped in 2d Scan Plot were saved as jpeg images. These images were imported into the SMAK image processing software package [53], where intensity was re-plotted on a log scale to better visualize the distribution of elements, and converted to greyscale.

X-ray energy at the beamline was controlled using an N_2-cooled Si(111) double-crystal monochrometer. The beam energy was calibrated using an Au foil placed below the beam path and above a caldiode solid-state detector. Part of the beam was deflected downward to excite the foil, and the absorption reading at the caldiode was normalized to the counts in an ion chamber upstream. The beam was focused by means of a 100 mm K-B mirror to approximately 5 μm.

Locations for XAS were chosen from the XRF microbeam maps, described above. At locations where the target metal(loid) appeared elevated on the map, a multichannel analyzer was employed to measure fluorescent X-ray intensity over a range of energies. Elements (atomic number Z>15) present at that location were identified by the energies of the emission peaks. At selected locations, XANES data were collected using a multielement Ge solid-state detector. Each detector element was set up to record the fluorescence intensity within the emission energy range corresponding to a target metal. Twelve detector elements were utilized for each of the contaminants (Cr, As, Pb), and their signals were summed to obtain the relevant XANES spectrum. The summed data was processed using the software Athena, as described above for the spectra collected at SSRL.

These data provided an improved conceptual understanding of the molecular-level speciation of Pb, Cd, Cr, and As in the soils, and how the molecular speciation influenced the resulting bioaccessibility. The metal speciation results were used to confirmed macroscopic observations of metal bioavailability for both the *in vitro* and *in vivo* methods.

More specifically, the geometric relationship between a metal and its nearest neighboring atoms were interpreted to indicate whether it was adsorbed onto a mineral surface or part of the internal mineral structure. This was accomplished by evaluating the identities, distances, and coordination numbers to atoms closely neighboring the metal by comparison of the EXAFS with theoretical phase and amplitude functions generated from postulated coordination chemistry scenarios.

A metal that is structurally incorporated into the mineral structure likely will not become bioavailable unless the mineral decomposes, whereas a metal that is adsorbed to a particle surface may be mobilized into the dissolved phase if chemical conditions change. For example, introduction of competing ions that can displace the adsorbed metal, a pH change, or a change in redox conditions can destabilize the metal-particle association. An outer-sphere association (electrostatic attraction) is generally less stable than an inner-sphere association (direct chemical bond).

Both As and Cr exhibit multiple potential oxidation states that influence their toxicity. Dissolved As(III) is typically more toxic than As(V) and also has a lower affinity with mineral surfaces. For Cr, it is the oxidized form (Cr(VI)) that is more mobile and toxic than Cr(III). The oxidation states were easily distinguished from the XANES by the energy at which radiation was absorbed by an inner-shell electron. The absorption edge shifts to higher energy for oxidized species, and a characteristic pre-edge peak is associated with Cr(VI) [54]. The edge position and shape was also compared with that of mineral reference standards.

5.1.3 *In Vitro* Investigations to Assess Human Health Risks

OSU IVG: The OSU-IVG is a rapid, inexpensive and reliable screening tool for determining the potential bioavailability (i.e., bioaccessibility) of soil contaminants including As [37[. The OSU IVG method simulates important parameters of the human GI tract under fasting conditions. The amount of contaminant extracted by the OSU-IVG is assumed to be available for absorption across the intestinal membrane (i.e., bioaccessible) and incorporation into systemic circulation. Contaminant bioaccessibility is expressed as a percentage of the total contaminant content of the test sample. Two bioaccessibility values are determined by the OSU IVG: gastric and intestinal. For gastric bioaccessibility, 150 milliliters (mL) of gastric solution (0.10 molar [M] American Chemical Society [ACS] grade sodium chloride (NaCl) and 1% porcine pepsin, Sigma Aldrich, St. Louis, MO, Cat. No. P7000) is heated in an open extraction vessel, in a 37 degrees Celsius (ºC) hot water bath. When the solution reaches 37º C, the pH is adjusted to 1.8 ± 0.1 using 6 M trace metal grade hydrochloride (HCl) followed by addition of the soil (1 gram [g], < 250 micromoles [µm]). The sample is thoroughly mixed with the solution to maintain a homogenous suspension. The pH is continuously monitored and adjusted to 1.8 ± 0.1 for 1 hour (h). After 1 h, 10 mL of gastric solution is removed for analysis. The extract is immediately centrifuged (11,160 g for 15 minutes [min]) and then filtered (0.45 µm). Filtered extracts are refrigerated (4º C) for preservation prior to analysis. Intestinal bioaccessibility is determined from the gastric sample. The gastric sample is adjusted to 6.5 ± 0.1 using dropwise additions of a saturated sodium hydroxide (NaOH) solution followed by the addition of 0.563 g of porcine bile extract (Cat. No. B8631) and 0.563 g of porcine pancreatin (Cat. No. P1750 Sigma Aldrich, St. Louis, MO). The pH is continuously monitored and adjusted to 6.5 ± 0.1. After 2 h of mixing, 10 mL of intestinal

solution is collected for analysis. The extract is immediately centrifuged (11,160 g for 15 min) and then filtered (0.45 µm). Filtered extracts are refrigerated (4º C) for preservation prior to analysis. Three replicates analyses of soil test samples are performed to determine bioaccessible contaminants by OSU IVG. Extracts are analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES) or mercury (Hg)-ICP-AES. Calibration standards, check standards, and dilutions are prepared in 0.1 M ACS grade NaCl, and 0.5 M trace metal grade HCl matrix. A blank and a laboratory control sample are included with each batch of *in vitro* sample extractions for quality control.

PBET: The PBET developed by Ruby et al. [5, 32] was utilized at a variety of pH conditions to estimate metal bioaccessibility for a variety of stomach environments indicative of food intake, or lack thereof. Using the method of Stewart et al. [21, 22] additional soil property-driven models were constructed using the PBET method at these pH values. This is particularly important for Pb contaminated soils since Pb bioaccessibility decreases with an increase in pH [20, 36]. In contrast, As(V) bioaccessibility was minimally influenced by changing pH environments. Triplicate samples of 0.3 g dry soil are placed in 50 mL polyethylene tubes to which 30 mL 0.4 M glycine at pH 1.5 and 2.5 are added. The slurries are quickly placed in a rotating water bath of 37EC and agitated at 30 ± 2 revolutions per minute (rpm) for 1 h. After 1 h the samples are rapidly cooled in an ice bath. Supernatant is separated from the solid via centrifugation. The pH of the supernatant is measured to ensure that the final pH is within ± 0.5 pH units of the initial pH.

Metal bioaccessibility and metal bioavailability for the three study soils was calculated using soil property-driven models developed from our earlier studies. Calculated bioaccessibility values were compared with measured bioaccessibility values using *in vitro* gastrointestinal methods for study soils.

5.1.4 *In Vitro* Investigations to Assess Ecological Risks

Soil extraction methods: For ecological risk estimates, metal bioavailability was estimated from multiple regression and path analysis models developed using toxicity and bioaccumulation data from 26 soils (previous EPA-NCEA project). Additionally, 12 selected DoD sites (24 soils) from ER-1166 were tested in addition to the three soils used in the *in vivo* swine test. In the ecological investigations, data from *in vitro* DoD soil metal extraction coupled with DoD soil chemical and physical properties were compared to existing statistical relationships for estimating metal bioavailability to plants and soil invertebrates. Initially, statistical relationships developed for metal availability from a set of 26 soils were used to estimate the chemical availability of metals in DoD soils, based upon total metal levels and soil physical/chemical characteristics. This was followed by extraction of the DoD soils using extraction with several soil chemical extraction methods (e.g., pore water, dilute calcium nitrate and Mehlich 3 solution) [46, 47] to actually measure the chemical availability of metals in DoD soils. These measurements were compared to predicted chemical availability estimated by the models to determine the ability of the models to predict metal availability. The statistical models were used to predict the toxicity of the DoD soils to earthworms and plants, assuming additivity of the toxicity of individual metals. Although the various metals in a potential mixture have different modes of toxic action, it is difficult to make any other assumption than additivity of toxicity. However, we attempted to estimate Toxic

Units contributed by each metal to get an estimate of potential toxicity. Bioassays were conducted with DoD soils to determine actual toxicity and these results were compared to the model predictions. Comparison of the actual toxicity from bioassays with predicted toxicity from *in vitro* models was used to quantify the ability of *in vitro* models to predict actual ecotoxicity in field DoD soils. This served as the basis for validation of the *in vitro* methods for field DoD soils.

5.1.5 *In Vivo* Investigations

Plant: Plant bioassays with Perennial ryegrass, *Lolium perrene*; and Lettuce, *Lactuca sativa*, were conducted according to Dayton et al. [38-42] with contaminated soils from DoD to provide plant risk-based endpoints of germination, dry matter growth, and tissue metal concentrations. Metal uptake was monitored in both plant species weekly until a steady state was reached, prior to plant bioassays being performed.

Soil Invertebrate: Metal bioavailability and ecotoxicity in contaminated soils collected from DoD sites was assessed using soil invertebrate bioassays with earthworms (*Eisenia fetida*), potworms (*Enchytraeus crypticus*), and collembola (*Folsomia candida*) according to standard protocols [55, 56]. Bioassay endpoints included mortality, reproduction, and internal concentration of metals (bioaccumulation).

Swine: Metal bioaccessibility calculated by ER-1166 *in vitro* methods using DoD soils were correlated with metal bioavailability using *in vivo* immature swine dosing trials. The pig has been used as an animal model in a number of research fields including gastroenterology, nutrition, and metabolism. Specific justification for the use of swine in chemical bioavailability studies with soil matrices revolves primarily around biological (anatomical, physiological, biochemical) similarities to humans. There is an extensive database of information on the use of the swine model. Standard operating procedures (SOP) using the immature swine model developed by Dr. Stan Casteel, University of Missouri-Columbia Veterinary Medical Diagnostic Laboratory, have been approved by EPA Region 8 for measuring the bioavailability of Pb from incidental ingestion of soils by children. During the past 10 years, the swine model has served well as a surrogate for study of systemic bioavailability of soil Pb in a sensitive population of humans. More than 30 Superfund Site soils from locations across the nation have been tested. The swine model uses relative bioavailability data as measured by comparing oral absorption of the metal of interest in test soils to oral absorption of some fully soluble form of the metal. The fraction of the absorbed dose of a metal can be measured using concentrations in blood and tissues such as liver, kidney, and bone. For the special case of As, the urinary excretion fraction is most appropriate for estimating relative bioavailability. It has been shown by Weis *et al.* [57] that preliminary site-specific estimates of soil Pb relative bioavailability in 20 soils of concern to EPA ranged from 6% to greater than 85%, relative to the absorption measured for Pb from Pb acetate. The model has also been used successfully to assess the bioavailability of Cd and As.

A study using juvenile swine as test animals was performed to measure the GI absorption of Pb from a sample collected from the Portsmouth Naval Shipyard. The test material contained a Pb concentration of 4113 micrograms per gram (µg/g). The relative bioavailability of Pb in the sample was assessed by comparing the absorption of Pb from the test material to that of a

reference material (Pb acetate). Groups of five swine were given oral doses of Pb acetate or test material twice a day for 14 days. The amount of Pb absorbed by each animal was evaluated by measuring the amount of Pb in the blood (measured on days 0, 3, 7, 9, 12, and 15) and the amount of Pb in bone (measured on day 15 at study termination). The amount of Pb present in blood or bone of animals exposed to test material was compared to that for animals exposed to Pb acetate, and the results were expressed as relative bioavailability (RBA). RBA is the criterion that is most often used in risk assessments. It is derived by dividing the selected measure of bioavailability using the test material by the same selected measure of bioavailability using a reference material known to be highly bioavailable. Careful attention should be paid to the derivation of RBA or predicted RBA, as the results of this study show that the most accurate approach will be site specific.

A study using juvenile swine as test animals was performed to measure the GI absorption of As from a soil sample taken in the vicinity of the Deseret Chemical Depot. The soil sample contained an As concentration of 521 µg/g. The relative bioavailability of As was assessed by comparing the absorption of As from the test material to that of a reference material (sodium arsenate). Groups of five swine were given oral doses of sodium arsenate or the test materials twice a day for 14 days; a group of three non-treated swine served as a control. The amount of As absorbed by each animal was evaluated by measuring the amount of As excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The urinary excretion fraction (UEF) (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and sodium arsenate using linear regression analysis. The RBA of As in the test soil compared to that in sodium arsenate was calculated as follows:

$$RBA = \frac{UEF\ (test\ soil)}{UEF(sodium\ arsentae)}$$

A study using juvenile swine as test animals was performed to measure the GI absorption of chromium from a soil sample taken in the vicinity of McClellan AFB. The soil sample contained a Cr concentration of 593 µg/g. The relative bioavailability of Cr was assessed by comparing the absorption of Cr from the test material to that of a reference material (Cr chloride). Groups of five swine were given oral doses of Cr chloride or the test materials twice a day for 14 days; a group of three non-treated swine served as a control. The amount of Cr absorbed by each animal was evaluated by measuring the amount of Cr excreted in the urine (collected over 48-hour periods beginning on days 6, 9, and 12). The UEF (the ratio of the amount excreted per 48 hours divided by the dose given per 48 hours) was calculated for both the test soil and Cr chloride using linear regression analysis. The RBA of Cr in the test soil compared to that in Cr chloride was calculated as follows:

$$RBA = \frac{UEF\ (test\ soil)}{UEF(chromium\ chloride)}$$

Statistics:

The ability of bioaccessibility to predict bioavailability. Measured bioaccessible Pb and As for DoD test soils was inserted into previously published regression equations used to predict Pb bioavailability [58] and to predict As bioavailability [19, 37, 59]. Predicted bioavailability was

compared with the measured 90% confidence interval for Pb and As bioavailability from swine dosing trials.

The ability of soil properties to predict bioaccessibility. Measured soil properties for DoD test soils was inserted into previously published regression equations used to predict As bioaccessibility [20, 60] and to predict Cr bioavailability [21, 22]. The RMSE for predicted-actual bioaccessibility values was used to determine the ability of soil properties to predict As or Cr bioaccessibility.

The ability of soil properties to predict metal bioavailability to plants. Statistical models were developed using soil property and plant uptake data from a combined NCEA and SERDP database. Both multiple linear regression (MLR) and ridge regression (RR) models were developed. The developed models were evaluated to determine their ability to predict metal bioavailability to plants for the ESTCP study soils. Both types of models were fit to the data using PROC REG in SAS 9.2. For the MLR models, model selection was not performed; we included all five independent variables (pH, OC, FEAL, CEC, and Total) in each model. For the RR models, an extra penalty term is added to the statistical model. This penalty term can be tuned to adjust the parameter estimates, increasing the bias in the parameter estimates while decreasing the influence of multicollinearity on the parameter estimates. These biased estimates produce a model that does not fit the observed data as closely as the MLR. In all cases, the R^2 for the MLR will be superior to the one obtained from the RR. However, the biased estimates produced by the RR often produce a better predictive model, and that was the central goal of our model development.

When using the RR approach, we chose the value of the tuning parameter by selecting the value that minimizes the PRESS statistic. The PRESS statistic is calculated by removing each observation, in turn, from the dataset; fitting the model using the remaining n – 1 observations; using the model fit to obtain a predicted value for the removed observation; and calculating the squared error of prediction for the removed observation. After cycling through each observation in the dataset in this manner, the squared errors of prediction are summed to obtain the final PRESS statistic. The model with the lowest PRESS statistic is declared to have the best predictive ability. Predicted residual sum of squares (PRESS) statistics cannot be compared between RR models with different dependent variables, and there isn't a specific value of the PRESS statistic that can be considered adequate for declaring a model to have good predictive ability. However, the PRESS statistic can be used to compare two or more RR models with the same dependent variable.

The ability of soil extraction methods to predict metal bioavailability to plants. Regression models developed using bioaccumulation data from the NCEA study were used to predict contaminant phytoaccumulation in the study soils. Comparison of the actual contaminant phytoaccumulation from bioassays with predicted phytoaccumulation from soil extraction methods were used to quantify the ability of soil extraction models to predict actual phytoaccumulation in field DoD soils.

5.2 BASELINE CHARACTERIZATION

Key observations from the synchrotron X-ray studies are (1) Pb is present as adsorbed divalent ions or as organic complexes, rather than in crystalline compounds, in all of the Pb-rich soil samples; (2) Cr is present as Cr(III), the more stable and less toxic of the two common Cr oxidation states, in all three Cr-rich soil samples; and (3) As is present in the more stable and less toxic form, As(V), in three of the four As-rich soil samples, but is present as both As(III) and As(V) in the sample from the Naval Complex at Pearl Harbor. As appears to occur as an adsorbed complex on iron oxides in the Concord and Pearl samples, and as an adsorbed complex on aluminum oxides in the Hilo soil sample. No Pb was found to be bound in more immobile and less bioaccessible sulfide phases, meaning that most of the Pb-O in the soils can be liberated under acidic conditions (i.e., in the stomach or in the case of percolating acidic soil/groundwater). The finding that Pb is mobilizable in low pH conditions is supported by previous flow-through and leaching experiments performed on the Cherry Point soils [61]. Please see Appendix A of the Final Report for detailed baseline characterization.

5.3 TREATABILITY OR LABORATORY STUDY RESULTS

Please see Appendices A through F of the Final Report for detailed study results.

5.4 DESIGN AND LAYOUT OF TECHNOLOGY COMPONENTS

There were no technology components deployed in the field.

5.5 FIELD TESTING

The nominal project schedule is shown in Table 6. Investigation-derived wastes (IDW) were disposed of onsite at the individual Principal Investigator's laboratories. No field equipment was deployed or left in place.

Table 6. Project schedule.

	Year 1	Year 2	Year 3
Workshop with regulators, EPA, scientists, end users			
Prepare State of the Science and Regulatory Acceptance White Paper			
Prepare site selection memorandum and Draft and final Demonstration Plan			
Identify sites, collect and characterize soil			
Quantify *in vitro* bioaccessibility			
Quantify *in vivo* bioavailability			
In vivo ecological bioassays (plant/invert)			
In vivo swine dosing trials			
Metal speciation with XAS			
Model validation			

5.6 SAMPLING METHODS

Please see Appendices A through F of the Final Report for detailed sampling methods.

5.7 SAMPLING RESULTS

Please see Appendices A through F of the Final Report for detailed sampling results.

6.0 PERFORMANCE ASSESSMENT

The technical objectives of the investigation were: (1) To provide validation that the relationships between soil properties and *in vitro* bioaccessibility methods can serve as a screening tool for estimating *in vivo* toxic metal bioavailability in DoD soils; (2) To provide DoD with a scientifically and technically sound method for estimating human and ecological risk associated with metal contaminated soils in place of or as justification for more-detailed, site-specific bioavailability (e.g., animal dosing), and (3) to promote the use of *in vitro* methods in human health and ERAs through the upfront involvement of end-users and regulators and the subsequent dissemination of the results of the study in peer-reviewed journals.

Performance Objectives 1 and 2 involved testing the bioavailability screening tools developed in our earlier SERDP studies, which correlate chemical speciation, bioaccessibility, bioavailability, and toxicity of metals (Pb, As, Cd, Cr) in DoD soils as measured by biological models used to evaluate ecological risk (e.g., plants, earthworms) and human risk (e.g., immature swine model). Only three sites were considered for the *in vivo* swine dosing studies due to the experimental cost. The use of *in vitro* ecological models were further verified by comparison with *in vivo* ecological bioassay studies of eleven DoD soils (eleven contaminated, eleven control).

An important first step was characterizing the molecular-level speciation of the metals in the soil with the use of XAS. Synchrotron XRF microprobe mapping, microbeam XAS, and bulk sample XAS were used to determine the oxidation state and molecular coordination environment of As, Pb, and Cr in eleven study soils with variable soil properties. *In vivo* swine dosing trials to determine metal bioavailability, *in vitro* GI studies to determine metal bioaccessibility, soil extraction procedures and soil properties used to predict metal bioavailability to plant and soil invertebrates and ecological bioassay studies were also performed on the same set of soils. Findings from synchrotron X-ray studies indicated that Pb is adsorbed as divalent ions or present as organic complexes, rather than in crystalline compounds. Chromium and As are present in their more stable and less toxic inorganic forms, Cr(III) and As(V), except in soil from the Naval Complex at Pearl Harbor, where both As(III) and As(V) are present. Arsenic is bound to iron oxides in the Concord and Pearl samples, and to aluminum oxides in the Hilo soil sample. As-bearing soils may require more site-specific approaches to remediation. Pb was not bound in sulfide phases that would be considered stable, meaning that most of the Pb-O in the soils may be liberated under acidic conditions (i.e., in the stomach).

Metal bioaccumulation and toxicity to soil invertebrates (*E. andrei, En. crypticus, F. candida*) were examined in ESTCP metal-contaminated soils (with paired reference site soils) comprising a wide range of physical and chemical characteristics and metal levels. The predictive ability of a number of different models relating soil properties to oligochaete metal bioaccumulation and toxicity as a screening tool for estimating metal bioavailability in soils was examined with the intent of validating some of these models for predicting metal bioaccumulation in soil-dwelling oligochaetes.

Key elements for predicting bioaccumulation of metals by soil invertebrates include metal concentration in the soil, soil physicochemical characteristics, and time. In this study, we examined the application of various models, with varying degrees of success, in predicting the bioaccumulation of metals by earthworms from ESTCP soils. The models can be divided into three categories: 1) Metals for which a large number of models exist in the literature (e.g., Pb,

Cd); 2) Metals for which few models exist in the literature (e.g., Cr, Ni); and, 3) Essential metals (e.g., Cu, Zn).

When applying literature-based metal bioaccumulation models to assess Cd and Pb bioaccumulation by earthworms in metal-contaminated field soils, 98% of the variability in earthworm Cd concentrations could be predicted by a model comprising total soil Cd, organic matter content, and soil pH, while 95% of the variability in earthworm Pb concentrations could be predicted by a model including total soil Pb and soil pH. However, both these models over-predicted metal bioaccumulation (Cd 106%; Pb 272%) so their use in predicting bioaccumulation may be limited. A large portion of the variability in the tissue concentrations of As (90%), Cr (77%), and Ni (88%) could be estimated by their concentrations in soil. Even though just a few bioaccumulation models exist for these metals, the models for As (24.2%) and Cr (13.6%) provided acceptable predictions of metal uptake, while the Ni model severely over-predicted uptake (689%). However, for the essential metals Cu and Zn, total soil concentrations combined with soil properties provided a reasonable prediction of tissue concentrations for Cu (24.7%) but not for Zn (590%). A model relating bioaccumulation factor (BAF) of Cd to soil properties provided acceptable predictions of Cd BAFs by *En. crypticus* from ESTCP soils (20%) while no relationship was evident between BAFs and observed metal burdens for Pb and Zn.

Models developed relating 0.5 M $Ca(NO_3)_2$-extractable Cd and Pb to earthworm metal residues did not provide a better prediction of Cd and Pb concentrations in earthworms exposed to ESTCP soils than models selected from the literature that predicted earthworm metal concentrations based upon total metal levels and soil physicochemical characteristics. Models incorporating toxicokinetics of metals were only available for Cd and provided reasonable estimates of Cd concentrations in earthworms (19%). These results indicate that there are no models for a specific metal that would provide good predictions of metal bioaccumulation in all soils and situations.

Contaminant phytoaccumulation was also determined from plant bioassays for soils from eleven study sites. For ecological risk estimates, metal phytoavailability was estimated from soil-property driven multiple regression models developed using bioaccumulation data from two previous study studies. A separate approach involved the use of soil extraction methods, used to estimate metal(loid) phytoavailability, to predict contaminant phytoaccumulation. Regression models developed using bioaccumulation data from a previous study sponsored by NCEA were used to predict contaminant phytoaccumulation in the study soils. Comparison of the actual contaminant phytoaccumulation from bioassays with predicted toxicity from *in vitro* models were used to quantify the ability of *in vitro* models to predict actual phytoaccumulation in field DoD soils. This was the basis for validation of the soil property or soil extraction methods for field DoD soils. The predictive capability required by a soil property/soil extraction models depends on the degree of accuracy of contaminant phytoaccumulation determined by the risk assessor. With some exceptions, both methods were able to predict phytoavailability at <35% of the measured contaminant tissue value. In general, soil property models were predictive of tissue As, Cd, and Pb. Exceptions were Deseret for As (ryegrass), Hill for Cd (lettuce), and Portsmouth for Pb. In general, the predictive capability of soil extraction methods was adequate to excellent with the exception of Hill for Cd (lettuce) and Portsmouth for Pb.

Table 7. Summary of the prediction of metal bioaccumulation by earthworms (*Eisenia fetida*) or potworm (*Enchytraeus crypticus*) using soil property or soil extraction data.

Approach	Metal	Model	Summary and Ability to Predict Metal Body Burdens
Soil Properties	As	$\ln As_{ew}=0.9884*\ln As_s - 1.747$ Sample et al. 1998	Based on total As levels; $R^2=0.90$; under predicts 0.8-16-fold, most soils 0.8-3.3 fold; RMSE = 24.2%
	Cd	$\ln Cd_{ew} = 6.018 + 0.787 * \ln Cd_s - 0.106 *OM - 0.402 * pH$ Ma et al. 1983	Based on total Cd, organic matter, pH; $R^2=0.98$; over predicts 3.8-11.3-fold; only eight data points above DL; RMSE = 106%
	Cr	$\log Cr_{ew}=0.69*\log Cr_s -1.05$ Peijnenburg et al. 1999a	Based on total Cr; $R^2=0.73$; under predicts 0.8-7.4-fold; RMSE == 13.6%
	Cu	$\log Cu_{ew}=0.435*\log Cu_s +0.39$ Morgan and Morgan 1988	Based on total Cu; $R^2=0.45$; under predicts 1.3-5.2-fold; RMSE = 24.7%
	Ni	$\log Ni_{ew}=0.98*\log Ni_s +0.67$ Neuhauser et al. 1995	Based on total Ni; $R^2=0.88$; over predicts 11-95-fold; RMSE = 689%
	Pb	$\log Pb_{ew} = 2.65+0.897 *\log Pb_s-3.56*\log pH$ Corp and Morgan 1991	Based on total Pb and pH; $R^2=0.95$; over predicts 0.5-25-fold; RMSE = 272%
	Zn	$\log Zn_{ew}=1.45*\log Zn_s +0.42$ Peijnenburg et al. 1999a	Based on total Zn; $R^2=0.62$; under predicts 1.3-5.2-fold; RMSE = 590%
	Cd	$C_w = 9.32 *e^{-0.008*28} + Cd_s *0.052/0.008*(1- e^{-0.008*28})$ Yu and Lanno 2010	Based on Cherry Point and McLellan soils where total Cd is same as model concentration, one prediction is the same as observed and one is 2-fold higher; with all 8 data points –RMSE = 19%
Calcium Nitrate Extraction	Cd	$\log Cd_{ew} = 0.27*\log Cd_{Ca(NO3)2} + 2.1$ $R^2 = 0.66$,	Only two soils – Cherry Point, McLellan – with total extractable Cd levels; over predicted earthworm Cd 3-6.8-fold; RMSE = 111%
	Pb	$\log Pb_{ew} = 0.32\ Pb_{Ca(NO3)2} + 97$ $R^2 = 0.39$, P=0.008	Only five soils with extractable Pb; over predicted 1.1-3.6-fold; RMSE = 161%
	Zn	$\log Zn_{ew} = 0.02\ Zn_{Ca(NO3)2} + 2.12$, $R^2=0.084$, P=0.21	Only four soils with extractable Zn; under predicted 1.3-2-fold; RMSE = 101%
BAF - Soil Properties *En.crypticus*	Cd	log BAF=1.17-0.92*log Clay Peijnenburg et al. 1999b	Only six soils where BAF could be calculated; acceptable under-prediction; RMSE = 21%
	Pb	log BAF=0.35-0.36*pH Peijnenburg et al. 1999b	No relationship
	Zn	$\log BAF =3.47-0.46 *pH-0.67*\log Al_{ox}$ Peijnenburg et al. 1999b	No relationship

DL = detection limit

The predictive capability of soil property/soil extraction models to predict plant phytoaccumulation is summarized as follows.

Table 8. Summary of the prediction of contaminant phytoaccumulation using soil property or soil extraction soil data

Approach	Model or Soil Extraction	Ability to Predict Tissue As		Ability to Predict Tissue Cd		Ability to Predict Tissue Pb	
		Lettuce	Ryegrass	Lettuce	Ryegrass	Lettuce	Ryegrass
Properties	MLR	4† Concord Over , 5x‡	4 Deseret Over, 80x	4 Hill Under, 1.7x	4	7 Portsmouth Over, 1.3x ORNL Under, 1.3x	7 Portsmouth Under, 1.2x
	RR	4	4 Deseret Over, 80x	4 Hill Under, 1.7x	4	7 Portsmouth Over, 2x ORNL Over, 2x	7 Portsmouth Over, 1.7x
Soil Extraction	Pore water	3	3 All sites Over, 2x	3	3 Hill Under, 1.6x	4 Portsmouth Under, 4x	4 Portsmouth Under, 3.3x
	Mehlich 3	4	4 all sites Over, 2x to 5x	NA	NA	NA	NA
	Calcium Nitrate	NA	NA	3 Hill Under, 10x	3 Hill Under, 4x	4 Portsmouth Under, 2x	4 Portsmouth Under, 2.5x

† Number of contaminated soils evaluated.
‡ Over prediction of tissue As concentration by a factor of five

One of the main objectives of the project was to determine the ability of *in vitro* GI methods (i.e., bioaccessibility methods) to predict measured contaminant bioavailability in contaminated soils from study sites. Equations used to predict bioavailability from bioaccessibility methods are available for Pb and As.

Relative bioavailable Pb was determined for the Portsmouth soil in our study. The PBET methods (pH 1.5 and 2.5) were able to accurately predict *in vivo* RBA for the Portsmouth soil. The predicted RBA for the PBET method at pH 2.5 was closer to actual *in vivo* RBA than pH 1.5. However both methods predict RBA Pb within the 90% confidence interval. The OSU IVG method In vitro Bioaccessibility (IVBA) Pb was very close to the *in vivo* RBA Pb. However, information on the ability of the OSU IVG method to predict RBA Pb is very limited whereas in depth validation studies have been conducted for the relative bioaccessibility leaching procedure (RBALP) (i.e., PBET) method. These results support the use of the PBET method at pH 1.5 and 2.5 to accurately predict *in vivo* RBA Pb. Future validation studies where this approach is expanded from the Portsmouth soil to other DoD soils will increase the confidence of using *in vitro* methods to predict *in vivo* RBA Pb.

Table 9. Comparison of measured and predicted RBA Pb for the Portsmouth soil.

Measured Pb RBA, %		Predicted Pb RBA				OSU IVG pH 1.8
		PBET pH 1.5		PBET pH 2.5		
Mean	90 % CI†	IVBA, %	RBA, %	IVBA, %	RBA, %	IVBA, %
99	70 - 127	83.3	86.9	80.4	106.2	102.5

† CI = confidence interval

Results from our study show both the OSU IVG and PBET method were able to predict RBA As in the Deseret soil. The predicted RBA As by all methods ranged from 12.2 % to 16.2%, which is comparable to the *in vivo* RBA As of 14%. Further validation studies of these methods for other contaminated soils from different DoD contaminant sources are warranted. A study investigating the relationship between *in vitro* IVBA Cr and *in vivo* RBA Cr has not been reported. Thus, it was not possible to evaluate the ability of bioaccessible Cr to predict *in vivo* RBA Cr. In our study, a novel immature swine dosing model was used to determine the *in vivo* RBA Cr for the McClellan soil. RBA Cr was 107% with a 90% confidence interval ranging from 76% to 169%. *In vitro* IVBA Cr PBET method, used to measure bioaccessible Cr at pH 1.5 and at pH 2.5, was 10.1% and 19.0%, respectively. The *in vitro* IVBA values were much lower than the *in vivo* RBA Cr. Further research is needed before IVBA can be used to predict *in vivo* RBA Cr.

Table 10. Comparison of measured and predicted RBA As for the Deseret soil.

Measured As RBA, %		Predicted As RBA					
		OSU IVG gastric		OSU IVG intestinal		SBET gastric	
Mean	90 % CI†	IVBA, %	RBA, %	IVBA, %	RBA, %	IVBA, %	RBA, %
14	13-15	8.45	15.0	8.47	16.2	10.6	12.2

† CI = confidence interval
SBET = Simplified Bioaccessibility Extraction Test

In general, all of the in vitro methods predicted in vivo RBA As with 90% confidence.

Studies of the determination of soil properties on *in vivo* bioavailability or IVBA are very limited. To our knowledge, these relationships have not been reported for Pb and limited studies exist for As and Cr. Key soil physical and chemical properties (e.g. particle size, CEC, Fe-oxides, TOC/TIC, pH) were identified as controlling the extent of toxic metals bioaccessibility as measured using the PBET that simulated the digestive system of humans. The bioaccessibility results (*in vitro*) were found to be in excellent agreement with molecular-level metal speciation studies, which confirmed that key soil properties control metal bioavailability.

The ability of soil properties to predict As and Cr bioaccessibility (IVBA) was dependent on the contamination source. In general, IVBA As measured by PBET and OSU IVG could be predicted from measured soil Fe properties including Feox or CBD Fe for soils where arsenical pesticide was the contaminant source. However, soil properties of the Deseret soil, where mine tailing was the contaminant source, was not predictive of the measured IVBA As. This finding suggests As may occur as discrete minerals from the mining operation. It is likely the insoluble As minerals in the mining waste did not appreciably dissolve and react with soil components.

Therefore, its chemical speciation and IVBA solubility will depend on the mining waste mineral not soil property.

The ability of soil properties (i.e., clay, organic and inorganic carbon) to predict and Cr bioaccessibility (IVBA) was dependent on the contamination source. Good agreement between the measured IVBA Cr and predicted IVBA Cr was found for Hill and McClellan soils. Poor agreement between the measured IVBA Cr and IVBA Cr predicted by soil properties was found for the Cherry Point soil. Differences in Cr chemical speciation in soil may offer an explanation. Water or wastewater treatment was the contaminant source for the Hill and McClellan soils. Incinerator ash was the contaminant source for the Cherry Point soil.

6.1 SUMMARY OF SOIL PROPERTIES TO PREDICT METAL BIOAVAILABILITY

Soil properties, able to predict metal (bio)availability for several contaminated soils in this study, are summarized in the following table. At a minimum, soil property information needed from a site investigation for all contaminants studied are soil pH, clay content, organic carbon (C), inorganic C, reactive Fe and Al (FEAL, Feox and/or CBD Fe). Other properties not studied that will affect ecological endpoints include soil salinity and the presence of other toxicants.

Table 11. Summary of soil properties to predict metal bioavailability.

	Contaminant			
	Pb	**As**	**Cr**	**Cd**
Human Soil Ingestion Bioaccessibility	Not evaluated	Feox and FeCBD	Clay content, total organic C, inorganic C	Not evaluated
Plant accumulation Lettuce	pH, OC, FEAL	pH, OC, FEAL	Not evaluated	pH, OC, FEAL
Plant accumulation Ryegrass	pH, OC, FEAL	pH, OC, FEAL	Not evaluated	pH, OC, FEAL
Soil Invertebrates	pH	Total metal	Total metal	pH, OM

These properties will **not** predict metal bioavailability for all soils. A major finding of this study is the contaminant source and likely speciation greatly affects the ability of soil property to predict metal bioavailability. Metal bioavailability was not able to be predicted for several soils where the contaminant source was unweathered mining waste or discrete inorganic mineral forms such as coal ash. Soil properties should NOT be used to predict contaminant bioavailability in these soils. More research on contaminant source and speciation is needed to determine when soil properties can provide an accurate assessment of metal bioavailability. Currently research is in progress, including research funded by SERDP (i.e., ER-1742) to determine the relationship between As speciation and ability to predict As bioavailability to humans.

6.2 SUMMARY OF SOIL EXTRACTION METHODS TO PREDICT METAL BIOAVAILABILITY

Soil exaction methods, able to predict metal (bio)availability for several contaminated soils in this study, are summarized in the following table. Both PBET and OSU IVG were able to very accurately predict RBA As and Pb but for only for 1 soil each. The number of soils evaluated were very limited because of cost constraints associated with in vivo dosing trails required to measure contaminant RBA. More research is needed to evaluate the ability of these methods to predict RBA Pb and RBA As on other contaminated soils.

Table 12. Summary of soil extraction methods to predict metal bioavailability.

	Contaminant			
	Pb	**As**	**Cr**	**Cd**
Human Soil Ingestion Bioaccessibility	PBET, pH 1.5 PBET, pH 2.5 OSU IVG	OSU IVG PBET	Not evaluated	Not evaluated
Plant accumulation Lettuce	Pore water 0.1 M $Ca(NO_3)_2$	Pore water Mehlich 3	Not evaluated	Pore water 0.1 M $Ca(NO_3)_2$
Plant accumulation Ryegrass	Pore water 0.1 M $Ca(NO_3)_2$	Pore water Mehlich 3	Not evaluated	Pore water 0.1 M $Ca(NO_3)_2$
Soil Invertebrates	Pore water 0.5 M $Ca(NO_3)_2$	Not evaluated	Not evaluated	Pore water 0.5 M $Ca(NO_3)_2$

Soil pore water was able to predict plant tissue concentration of Pb, As, and Cd. Soil extraction with 0.1 M $Ca(NO_3)_2$ was able to predict cationic metal contaminants(i.e. Pb, Cd) but was not evaluated for anionic As contamination. The ability of simply water or dilute calcium nitrate to predict phytoavailable contaminant suggests high solubility of these contaminants in soils. Thus, it is likely that with 0.1 M $Ca(NO_3)_2$ would have also been a good predictor of plant As. However, two cautions should be heeded. The accuracy of these extraction methods to predict plant tissue contamination was limited to ± 35%. Similarly to metal bioaccessibility results, metal bioavailability was not able to be predicted for several soils where the contaminant source was unweathered mining waste (i.e. Deseret) or discrete inorganic mineral forms such as coal ash (i.e. Cherry Point). Soil extraction methods listed in Table 12 should NOT be used to predict contaminant bioavailability in these soils. More research on contaminant source and speciation is needed to determine which soil extraction methods can provide an accurate assessment of metal bioavailability.

As part of Objective 3, immediately upon receiving funding for this endeavor, a two-day workshop was held bringing together state regulators, DoD site end users, EPA officials, and scientists familiar with soil metal bioavailability. The workshop focused on past, current, and future research endeavors investigating soil metal bioavailability methodologies and the possible use of *in vitro* bioaccessibility values in human health risk assessment and policy. At the kickoff workshop, the research strategy was discussed among scientists, regulators, EPA, and end-users to advance the acceptance of *in vitro* methods in human health and ERA and policy. We incorporated the comments of the attendees of the workshop in our research. In addition, also as part of Objective 3, most of the technical objectives, methods, results, discussion, conclusions, and recommendations of this study are detailed in Appendices A-F of the Final Report, which

were written as stand-alone manuscripts for submission as peer-reviewed publications. Publication in peer-reviewed journals is needed to disseminate and ultimately facilitate the results of this study by site managers. In addition, publication in peer-reviewed literature is crucial to ensuring regulatory and community understanding and acceptance of the scientific results. The publication of the results of this study are proceeding.

Please see Appendices A through F for the Final Report for a detailed performance assessment.

7.0 COST ASSESSMENT

Cost is an important part of the decision making process when doing bioavailability assessments and making risk management decisions. Questions a project manager must ask themselves include:

- How can I balance the cost of *in vivo* studies with the desire for reduced uncertainty when making risk assessment conclusions?
- What is the potential return on investment of a bioavailability study? Would adjustments to the RBA at the site lead to higher remedial goals? Would higher remedial goals allow for a reduced remedial footprint and reduced costs?
- Is there existing data that indicates reduced bioavailability of metals contaminants at the site?
- Does the project schedule allow for the time required to complete a bioavailability assessment?

The following sections provide cost information to help remediation professionals begin to answer these questions.

7.1 COST MODEL

The following tables provide simple cost model information. Site-specific bioavailability assessment will require a sampling and analysis plan, sample collection and reporting. These costs are estimated in Table 13. *In vitro* study costs are presented next in Table 14, followed by costs for the *in vivo* studies demonstrated in this study.

Table 13. Cost model for bioavailability assessment: sample collection and reporting.

Cost Element	Data Tracked During the Demonstration	Unit type: Number	Unit Cost	Estimated Costs
Sampling and analysis plan	• Personnel required and associated labor • Materials	Sampling and analysis plan document: 1	$8000	$8000
Sample collection and preparation	• Costs associated with labor and materials tracked	XRF: 1/sample Sample collection: 1/sample Grinding and sieving: 1/sample	$450/sample	$1350
Reporting	• Costs associated with labor tracked	Report documenting results of entire project: 1	$20,000	$20,000

Assumptions: Approximately 1 acre site with 3 samples. Sample collection and preparation includes necessary grinding and sieving for bioavailability studies.

Table 14. Cost model for bioavailability assessment: *in vitro* bioaccessibility.

Cost Element	Data Tracked During the Demonstration	Unit type: Number	Unit Cost	Estimated Costs
***In vitro* tests**	• Personnel required and associated labor • Analytical laboratory costs • Reporting	Set of three tests	$600 $110	$1800 $330
			Total	$2130

Assumptions: Each soil sample includes the following three replicate laboratory tests: reference, contaminated, and lab reference. Approximately 1 acre site with 3 samples. Sample collection and preparation includes necessary grinding and sieving for bioavailability studies.

Table 15. Cost model for bioavailability assessment: plant toxicity tests.

Cost Element	Data Tracked During the Demonstration	Unit type: Number	Unit Cost	Estimated Costs
Plant toxicity tests	• Personnel required and associated labor • Analytical laboratory costs	Lab technician, per unit cost (set of three tests/sample)	$3000	$9000
		Metals analysis and soil parameters	$500	$1500
Waste disposal	Hazardous waste or standard soil disposal			$200
Reporting			$55/hr	$275
			Total	$10,975

Assumption: Each soil sample includes the following three toxicity tests: reference, contaminated, and lab reference. Approximately 1 acre site with 3 samples. Sample collection and preparation includes necessary grinding and sieving for bioavailability studies.

Table 16. Cost model for bioavailability assessment: soil invertebrate toxicity tests.

Cost Element	Data Tracked During the Demonstration	Unit type: Number	Unit Cost	Estimated Costs
Soil Invertebrate toxicity tests – earthworm, potworm, and collembola	• Personnel required and associated labor • Analytical laboratory costs	Lab technician, per unit cost (set of three tests)	$4000 (Earthworm - $1200 Enchytraeid - $1200 Collembola - $1600)	$12,000
		Metals analysis and soil parameters	$500	$1500
Waste disposal	Hazardous waste or standard soil disposal			$200
			Total	$4700

Assumption: Each soil sample includes the following three toxicity tests: reference, contaminated, and lab reference. Approximately 1 acre site with 3 samples. Sample collection and preparation includes necessary grinding and sieving for bioavailability studies.

Table 17. Cost model for bioavailability assessment: in vivo swine study.

Cost Element	Data Tracked During the Demonstration	Unit type: Number	Unit Cost	Estimated Costs
Soil *in vivo* swine study	• Personnel required and associated labor • Analytical laboratory costs	Lab technician, per unit cost	$20,500	$61,500
		Animals/Supplies	$7500	$22,500
		Laboratory Analysis	$8500	$25,500
Waste disposal	Hazardous waste or standard soil disposal			$200
			Total	$109,700

All cost elements are provided on a per unit basis in the above tables. It is assumed that for the lower cost options such as an *in vitro* study, more samples could be analyzed leading to a broader understanding of RBA at the site.

7.2 COST DRIVERS

A site specific bioavailability analysis will vary in cost according to site specific factors that drive how many and what type of analysis is required. These variations in cost are apparent in the tables shown in Section 7.1. A significant driver in the determination of whether or not to pursue an adjustment of RBA is the potential cost avoidance.

Removal is the primary remedial technology available for soils contaminated with the metals studied. Soil removal, transportation and disposal costs for metal-contaminated soils can exceed $1000 per cubic yard. A significant reduction in remedial footprint can easily justify the expense of in vivo studies at some sites. An example is provided in Table 18 and Figure 1. This example shows an Hg-contaminated site where the initial remedial goal of 50 milligrams per kilogram (mg/kg) was based on the assumption that the Hg at the site was the soluble form $HgCl_2$ and was 100% bioavailable. Speciation and bioavailability studies were done and the risk assessment was revised based on the adjusted RBA of 10%. The final remedial goal for the site was 400 mg/kg reflecting an RBA of 10%, significantly reducing the footprint of the remediation area. The reduced footprint correlated with a more than 100,000 cubic yards (yd^3) reduction in soil volume to be removed and avoided almost $50 million in unnecessary remediation costs.

Table 18. Example bioavailability adjustment cost/benefit analysis.

Bioavailability (%)	Remediation Goal (mg Hg/kg soil)	1000 yd^3 excavated	Cost (10^6 1995 $)
100	50	120	81
30	180	54	49
10	400	10	34

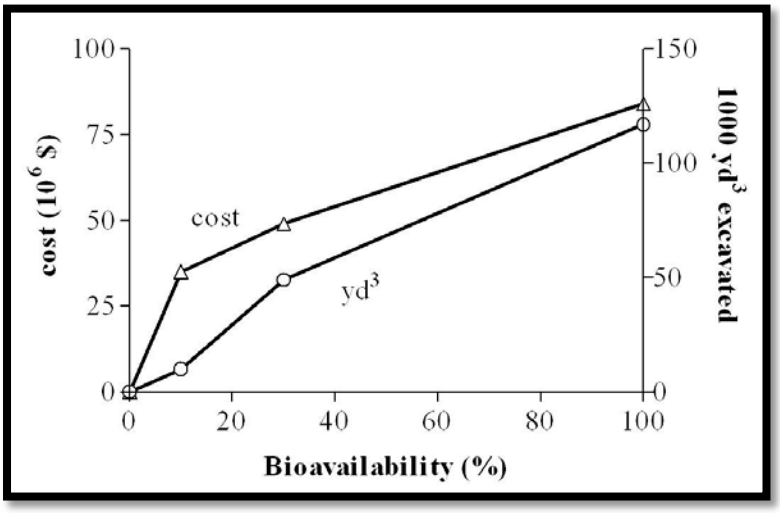

Figure 1. Example bioavailability adjustment cost/benefit analysis.

7.3 COST ANALYSIS

Consideration of cost should be part of the decision making process when determining whether bioavailability analyses are appropriate for a given site. Figure 2 provides a logical process to control costs related to bioavailability analysis. If metals concentrations in site soils indicate unacceptable risk using the HQ approach, a review of soil properties and current bioavailability assumptions should be done. If soil properties indicate that metals may be less bioavailable than assumed in the risk assessment, the next step towards adjusting the RBA is *in vitro* analysis. Before undertaking *in vitro* analysis consideration should be given to the site specific factors impacting the cost/benefit equation for the site. Factors that significantly affect whether or not a bioavailability study should be considered include: a) whether the studies can be completed within the required timeframe; b) the cost of the bioavailability study relative to cleanup; and c) whether or not existing data support the likelihood of reduced bioavailability.

If *in vitro* studies are completed and do indicate reduced RBA, the degree of certainty related to those adjustments should be documented for the project team. Understanding the results of the *in vitro* study in context can help the project team make the decision to use the results of the *in vitro* study in site risk assessment decisions. The team will also have the information necessary to determine if in vivo studies are required for making RBA adjustment decisions at the site and what the potential benefits of such studies are for the site.

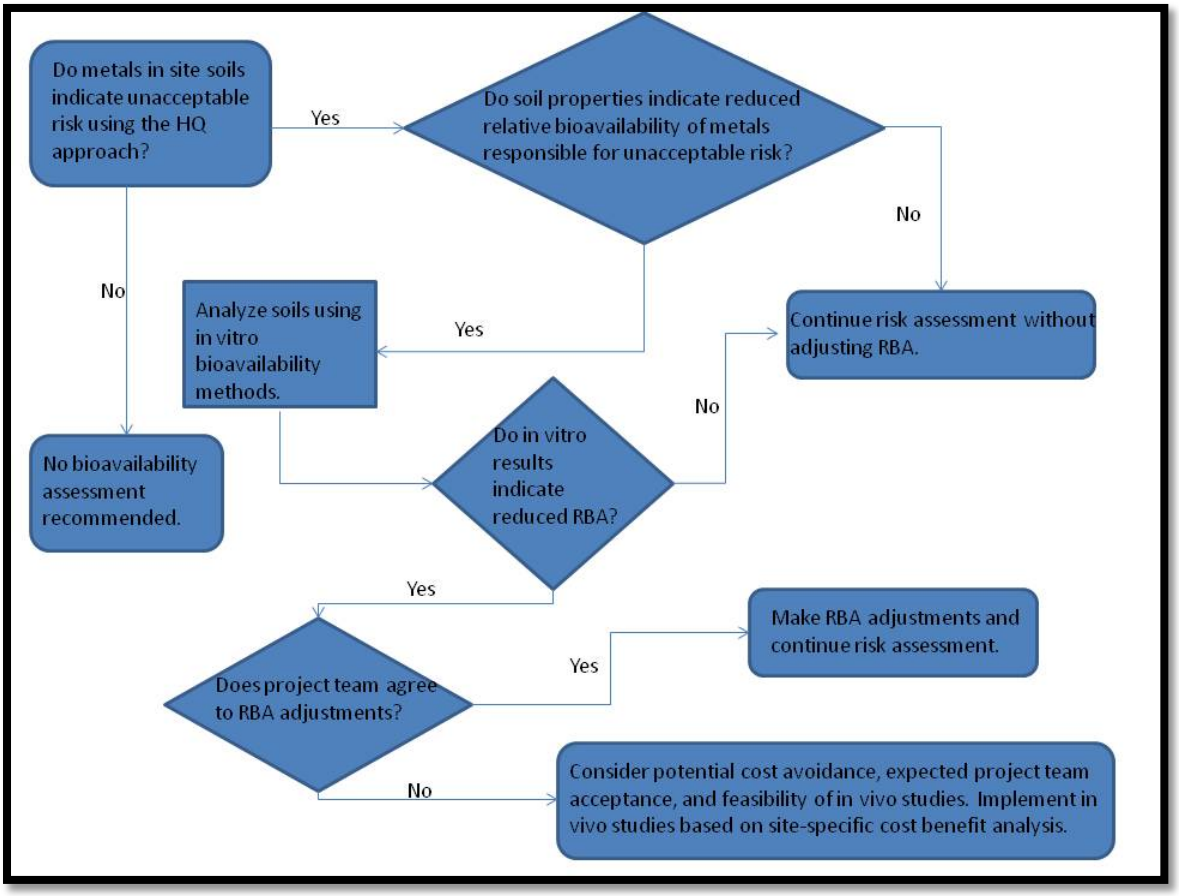

Figure 2. Process to control costs related to bioavailability analysis.

8.0 IMPLEMENTATION ISSUES

Results from this study show *in vitro* gastrointestinal methods can be used to predict bioavailable Pb and As via soil ingestion human exposure pathway. However the number of soils/sites were limited due to project costs. Further validation studies of these methods for other contaminated soils from different contaminant sources are warranted to increase acceptance of these methods in human health risk assessment by regulatory bodies. The ability of soil properties to predict bioavailability was inconsistent and contaminant source dependent. Soil properties were accurate predictors for some soil/contaminant source combinations but not others. Further studies are needed before a more detailed contaminant speciation model can be used to determine which soils may be suitable for estimating metal bioavailability using soil properties.

The predictive capacity afforded by soil property/soil extraction models depends to a large degree on the degree of accuracy of contaminant phytoaccumulation determined by the risk assessor. With some exceptions, both methods were able to predict phytoavailability at < 35% of the measured contaminant tissue value. In general, soil property models were predictive of tissue As, Cd, and Pb. Exceptions were Deseret for As (ryegrass), Hill for Cd (lettuce), and Portsmouth for Pb. In general, the predictive capability of soil extraction methods was adequate to excellent with the exception of Hill for Cd (lettuce) and Portsmouth for Pb.

In assessing the bioavailability and toxicity of metals in the soils of this study, it was apparent that soil invertebrates, particularly oligochaetes, exhibited reduced reproduction relative to the laboratory reference soil, in site reference soils. This was most extreme for earthworms, where reproduction in site reference soils was significantly lower in all but one site reference soil. Enchytraeid reproduction was lower in about half the site reference soils, while there was no effect of site reference soil on reproduction in Collembola. This suggests, that of the three soil invertebrates tests, earthworms are the least relevant since the soil types tested were unsuitable for earthworm reproduction regardless of whether elevated levels of metals were present. The reliance on earthworm testing of soils is widespread but may not be correct for certain soils, since *E. andrei* prefer soils rich in organic matter and reproduce poorly in soils with elevated sand or silt content. Enchytraeids are naturally found in a wider array of soils and can thrive in soils with a higher sand or silt content. Arthropods, such as Collembola, are affected even less by soil properties. The evaluation of metal bioavailability in soils with properties not conducive to testing with earthworms should incorporate tests using other soil invertebrates that are either indigenous to the soils being tested or which reproduce adequately in the test soils (e.g., enchytraeids, collembola, mites). In addition, soils found on DoD sites may be composites of soils that have been manually moved from a number of areas and deposited at sites distant from their origin. Additionally, many of these soils may not be suitable for earthworm inhabitation due to physical compaction, low moisture and organic matter content, and the presence of unmeasured chemicals. In short, the soils may be considered test substrates with unique properties, rather than actual soils, and warrant site-specific testing for chemical bioavailability and toxicity rather than assessment using standard extraction and chemical analysis.

EcoSSLs are conservative screening levels for contaminants in soil that are preferentially based upon toxicity data from soils where soil physical and chemical characteristics provide conditions of maximum chemical bioavailability. At least for soil invertebrates and plants, there does not

appear to be any clear relationship between toxicity and EcoSSL levels for metals in the DoD soils tested, as toxicity was observed in site reference soils as well as those where metal levels did not exceed EcoSSLs. Both field and laboratory research on evaluating the utility of EcoSSLs in site specific investigations is warranted.

Regulatory barriers for using bioavailability adjustments in ecological and human health risk assessments are complex and not easily resolvable. Regulatory acceptance of *in vitro* bioavailability in the near term will be on a case-by-case basis with most decisions based on site-specific data. Translating soil properties into field-scale risk assessment adjustments will also require consideration of future site uses that may alter soil characteristics and the subsurface environment and hence, bioavailability. This technical demonstration will contribute to this effort by providing significantly more complete and coupled data sets that link *in vivo* and *in vitro* bioavailability with soil characterization and metal speciation data.

The lack of guidance and policy coupled with time constraints on moving forward with cleanups present a regulatory barrier. The lack of guidance stems from insufficient published data to support the use of bioavailability adjustments in risk assessments. At present, *in vitro* data alone is generally not sufficient to make risk adjustments. More robust data sets are needed that correlate *in vitro* and *in vivo* data. Researchers must collect and publish data in peer-reviewed journals, including information on which *in vitro* tests work and which do not. Keeping regulators and site end-users abreast of these research findings will ultimately pave the way for an enhanced appreciation of *in vitro* methods as tools to estimate metal bioavailability on contaminated DoD sites. The ultimate publication of the results of this study will significantly help bridge this data gap. Publications and abstracts related to this study are described below in Table 19.

Table 19. Publication and abstracts.

Publications
Juhasz, A.L., N.T. Basta, and E. Smith. 2013. What is required for the validation of in vitro assays for predicting contaminant relative bioavailability? Considerations and criteria. Environmental Pollution 180:372-375.
Jardine, P.M., M.A. Stewart, M.O. Barnett, N.T. Basta, S.C. Brooks, S. Fendorf, and T.L. Mehlhorn. 2013. Influence of Soil Geochemical and Physical Properties on Chromium(VI) Sorption and Bioaccessibility. Environ. Sci. Technol. 47 (19):11241-11248
Yu, S., and R.P. Lanno. 2010. Uptake kinetics and subcellular compartmentalization of cadmium in acclimated and unacclimated earthworms (Eisenia andrei). Environ. Toxicol. Chem. 29:1568-1574.
Anderson, R.H., and N.T. Basta. 2009. Application of Ridge Regression to Determine the Effect of Soil Properties on Phytotoxicity of As, Cd, Pb, and Zn in Soil. Environ. Toxicol. Chem. 28:619-628.
Anderson, R.H., and N.T. Basta. 2009. Application of Ridge Regression to Quantify Marginal Effects of Collinear Soil Properties on Phytoaccumulation of As, Cd, Pb, and Zn. Environ. Toxicol. Chem. 28:619-628.
Anderson, R.H., N.T. Basta, and R.P. Lanno. 2008. Using a Plant Contaminant Sensitivity Index to Quantify the Effects of Soil Properties on Arsenate Phytotoxicity. J. Environ. Qual. 37:1701-1709.

Table 19. Publication and abstracts (continued).

Abstracts
Hawkins, A., N. Basta, E. Dayton, R. Lanno, M. Barnett, P. Jardine, S. Casteel, and K. Savage. 2009. Soil Properties, Metal Bioavailability and Risk Assessment. Partners in Environmental Technology Technical Symposium & Workshop sponsored by Strategic Environmental Research and Development Program (SERDP) and Environmental Security Technology Certification Program (ESTCP), Washington, DC. Dec 1-3, 2009.
Basta, N.T., S.D. Whitacre, E.A. Dayton, P.M. Jardine, J.S. Richey, S.W. Casteel, and A.L. Hawkins. 2011. Predicting Arsenic Bioavailability in Contaminated Soils Using Bioaccessibility or Soil Properties. 11th International Conference for Trace Element Biogeochemistry (ICOBTE), Florence, Italy. July 3-7, 2011.
Basta, N., E. Dayton, S. Whitacre, P. Jardine, S. Casteel, and A. Hawkins. 2011. Use of in Vitro or Soil Property Models to Assess Toxic Metal Bioavailability in Soil: Validation to Support Regulatory Acceptance. The 4th International Contaminated Site Remediation Conference, Adelaide, Australia, September 11–15.
Lanno, R.P., and S. Yu. 2010. Validation of laboratory models to predict metal bioaccumulation in earthworms using metal-contaminated field soils. SETAC 30th Annual Meeting, Portland, Oregon, November 7-11.
Yu, S., and R.P. Lanno. 2009. Uptake kinetics and subcellular compartmentalization of cadmium in acclimated and unacclimated earthworms (Eisenia andrei). SETAC 29th Annual Meeting, New Orleans, Louisiana, November 19-23.
Yu, S., and R.P. Lanno. 2009. The effect of soil properties on metal bioavailability to earthworms: Validation of laboratory models using metal-contaminated field soils. SETAC 29th Annual Meeting, New Orleans, Louisiana, November 19-23.
Yu, S., H. Anderson, N. Basta, and R.P. Lanno. 2008. The effect of soil properties on metal bioavailability to earthworms: Validation of laboratory models using metal-contaminated field soils. SETAC 29th Annual Meeting, Tampa Bay, FL, November 16-20.

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9.0 REFERENCES

Fendorf, Stanford University, unpublished data (p. 2)

Exponent. 2001. *Evaluation of the Metals that Drive Risk-Based Remedial Decisions at DoD Sites*. White Paper prepared for the Strategic Research and Development Program: Boulder, CO. p. 11.

Paustenbach, D.J. 1989. *A Comprehensive Methodology for Assessing the Risks to Humans and Wildlife Posed by Contaminated Soils: A Case Study Involving Dioxin., in The Risk Assessment of Environmental and Human Health Hazards: A Textbook of Case Studies*, D.J. Paustenbach, Editor. John Wiley & Sons: New York.

Sheehan, P.J., D.M. Meyer, M.M. Sauer, and D.J. Paustenbach. 1991. *Assessment of the Human Health Risks Posed by Exposure to Chromium-Contaminated Soils*. Journal of Toxicology and Environmental Health. **32**(2): p. 161-201.

USEPA. 1998. *Method 3050B: Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*. **SW-846** (Washington, DC, U.S. Environmental Protection Agency).

Ruby, M.V., R. Schoof, W. Brattin, M. Goldade, G. Post, M. Harnois, D.E. Mosby, S.W. Casteel, W. Berti, M. Carpenter, D. Edwards, D. Cragin, and W. Chappell. 1999. *Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment*. Environmental Science & Technology. **33**(21): p. 3697-3705.

Freeman, G.B., J.D. Johnson, S.C. Liao, P.I. Feder, A.O. Davis, M.V. Ruby, R.A. Schoof, R.L. Chaney, and P.D. Bergstrom. 1994. *Absolute Bioavailability of Lead Acetate and Mining Waste Lead in Rats*. Toxicology. **91**(2): p. 151-163.

Casteel, S.W., R.P. Cowart, C.P. Weis, G.M. Henningsen, E. Hoffman, W.J. Brattin, R.E. Guzman, M.F. Starost, J.T. Payne, S.L. Stockham, S.V. Becker, J.W. Drexler, and J.R. Turk. 1997. *Bioavailability of lead to juvenile swine dosed with soil from the Smuggler Mountain NPL site of Aspen, Colorado*. Fundamental and Applied Toxicology. **36**(2): p. 177-187.

Dieter, M.P., H.B. Matthews, R.A. Jeffcoat, and R.F. Moseman. 1993. *Comparison of Lead Bioavailability in F344 Rats Fed Lead Acetate, Lead-Oxide, Lead Sulfide, or Lead Ore Concentrate From Skagway, Alaska*. Journal of Toxicology and Environmental Health. **39**(1): p. 79-93.

Polak, J., E.J. Oflaherty, G.B. Freeman, J.D. Johnson, S.C. Liao, and P.D. Bergstrom. 1996. *Evaluating lead bioavailability data by means of a physiologically based lead kinetic model*. Fundamental and Applied Toxicology. **29**(1): p. 63-70.

Cheng, Y.L., J.E. Preslan, M.B. Anderson, and W.J. George. 1991. *Solubility and Bioavailability of Lead Following Oral Ingestion of Vitrified Slagged Aggregate*. Journal of Hazardous Materials. **27**(2): p. 137-147.

Preslan, J.E., C.Y. Chang, N.K. Schiller, and W.J. George. 1996. *Bioavailability of lead from vitrified slagged aggregate*. Journal of Hazardous Materials. **48**(1-3): p. 207-218.

Rieuwerts, J.S., and M.E. Farago. 1995. *Lead contamination in smelting and mining environments and variations in chemical forms and bioavailability*. Chemical Speciation and Bioavailability. **7**(4): p. 113-123.

Davis, A., J.W. Drexler, M.V. Ruby, and A. Nicholson. 1993. *Micromineralogy of mine wastes in relation to lead bioavailability, Butte, Montana*. Environmental Science and Technology. **27**(7): p. 1415-1425.

Ruby, M.V., A. Davis, J.H. Kempton, J.W. Drexler, and P.D. Bergstrom. 1992. *Lead Bioavailability - Dissolution Kinetics Under Simulated Gastric Conditions*. Environmental Science & Technology. **26**(6): p. 1242-1248.

Davis, A., M.V. Ruby, and P.D. Bergstrom. 1992. *Bioavailability of Arsenic and Lead in Soils From the Butte, Montana, Mining District*. Environmental Science & Technology. **26**(3): p. 461-468.

Yang, J.K., M.O. Barnett, P.M. Jardine, and S.C. Brooks. 2003. *Factors controlling the bioaccessibility of arsenic(V) and lead(II) in soil*. Soil and Sediment Contamination. **12**(2): p. 165-179.

Freeman, G.B., R.A. Schoof, M.V. Ruby, A.O. Davis, J.A. Dill, S.C. Liao, C.A. Lapin, and P.D. Bergstrom. 1995. *Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in cynomolgus monkeys*. Fundamental and Applied Toxicology. **28**(2): p. 215-222.

Davis, A., M.V. Ruby, M. Bloom, R. Schoof, G. Freeman, and P.D. Bergstom. 1996. *Mineralogic constraints on the bioavailability of arsenic in smelter-impacted soils*. Environmental Science & Technology. **30**(2): p. 392-399.

Rodriguez, R.R., N.T. Basta, S.W. Casteel, and L.W. Pace. 1999. *An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media*. Environmental Science & Technology. **33**(4): p. 642-649.

Yang, J.K., M.O. Barnett, P.M. Jardine, N.T. Basta, and S.W. Casteel. 2002. *Adsorption, sequestration, and bioaccessibility of As(V) in soils*. Environmental Science and Technology. **36**(21): p. 4562-4569.

Stewart, M.A., P.M. Jardine, M.O. Barnett, T.L. Mehlhorn, L.K. Hyder, and L.D. McKay. 2003. *Influence of soil geochemical and physical properties on the sorption and bioaccessibility of Cr(III)*. Journal of Environmental Quality. 32: p. 129-137.

Stewart, M.A., P.M. Jardine, C.C. Brandt, M.O. Barnett, S.E. Fendorf, L.D. McKay, T.L. Mehlhorn, and K. Paul. 2003. *Effects of contaminant concentration, aging, and soil properties on the bioaccessibility of Cr(III) and Cr(VI) in soil*. Soil and Sediment Contamination. **12**(1): p. 1-21.

Pierzynski, G.M., and A.P. Schwab. 1993. *Bioavailability of Zinc, Cadmium, and Lead in a Metal-Contaminated Alluvial Soil*. Journal of Environmental Quality. **22**(2): p. 247-254.

Krishnamurti, G.S.R., P.M. Huang, K.C.J. Vanrees, L.M. Kozak, and H.P.W. Rostad. 1995. *Speciation of Particulate-Bound Cadmium of Soils and Its Bioavailability*. Analyst. **120**(3): p. 659-665.

Sloan, J.J., R.H. Dowdy, M.S. Dolan, and D.R. Linden. 1997. *Long-term effects of biosolids applications on heavy metal bioavailability in agricultural soils*. Journal of Environmental Quality. **26**(4): p. 966-974.

Hamon, R.E., M.J. McLaughlin, R. Naidu, and R. Correll. 1998. *Long-term changes in cadmium bioavailability in soil*. Environmental Science & Technology. **32**(23): p. 3699-3703.

Luo, Y.M., and P. Christie. 1998. *Bioavailability of copper and zinc in soils treated with alkaline stabilized sewage sludges*. Journal of Environmental Quality. **27**(2): p. 335-342.

Boularbah, A., J.L. Morel, G. Bitton, and M. Mench. 1996. *A direct solid-phase assay specific for heavy-metal toxicity .2. Assessment of heavy-metal immobilization in soils and bioavailability to plants*. Journal of Soil Contamination. **5**(4): p. 395-404.

Chlopecka, A., and D.C. Adriano. 1996. *Mimicked in-situ stabilization of metals in a cropped soil: Bioavailability and chemical form of zinc*. Environmental Science & Technology. **30**(11): p. 3294-3303.

NEPI. 2000. *Assessing the bioavailability of metals in soil for use in human health risk assessment*. Washington, DC: National Environmental Policy Institute.

Schroder, J.L., N.T. Basta, J.T. Si, S.W. Casteel, T. Evans, and M. Payton. 2003. *In vitro gastrointestinal method to estimate relative bioavailable cadmium in contaminated soil*. Environmental Science & Technology. **37**(7): p. 1365-1370.

Ruby, M.V., A. Davis, R. Schoof, S. Eberle, and C.M. Sellstone. 1996. *Estimation of lead and arsenic bioavailability using a physiologically based extraction test*. Environmental Science and Technology. **30**(2): p. 422-430.

Ehlers, L.J., and R.G. Luthy. 2003. *Contaminant bioavailability in soil and sediment.* Environmental Science and Technology. **37**(15): p. 295A-302A.

Battelle and Exponent. 2000. *Guide for Incorporating Bioavailability Adjustments into Human Health and Ecological Risk Assessments at U.S. Navy and Marine Corps Facilities. Part 1: Overview of Metals Bioavailability.* Naval Facilities Engineering Service Center, Port Hueneme, CA.

Kelley, M.E., S.E. Brauning, R.A. Schoof, and M.V. Ruby. 2002. *Assessing Oral Bioavailability of Metals in Soil.* Columbus, OH: Battelle Press. 124.

Yang, J.K., M.O. Barnett, J.L. Zhuang, S.E. Fendorf, and P.M. Jardine. 2005. *Adsorption, oxidation, and bioaccessibility of As(III) in soils*. Environmental Science & Technology. **39**(18): p. 7102-7110.

Basta, N.T., J.N. Foster, E.A. Dayton, R.R. Rodriguez, and S.W. Casteel. 2007. *The effect of dosing vehicle on arsenic bioaccessibility in smelter-contaminated soils*. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering. **42**(9): p. 1275-1281.

Pedhazur, E.J. 1997. *Multiple Regression in Behavioral Research*. 3rd ed, Toronto, Canada: Wadsworth Thomson Learning.

Maruyama, G.M. 1998. *Basics of Structural Equation Modeling*, London: Sage.

SAS Institute, Release 8.022001, Cary, NC, USA.

ASTM. 2003. *Standard Guide for Conducting Terrestrial Plant Toxicity Tests, E 1963-02*, American Society for Testing and Materials, Philadelphia PA.

Dayton, E.A., N.T. Basta, M.E. Payton, K.D. Bradham, J.L. Schroder, and R.P. Lanno. 2006. *Evaluating the contribution of soil properties to modifying lead phytoavailability and phytotoxicity*. Environmental Toxicology and Chemistry. **25**(3): p. 719-725.

Bradham, K.D., E.A. Dayton, N.T. Basta, J. Schroder, M. Payton, and R.P. Lanno. 2006. *Effect of soil properties on lead bioavailability and toxicity to earthworms*. Environmental Toxicology and Chemistry. **25**(3): p. 769-775.

Heuscher, S.A., C.C. Brandt, and P.M. Jardine. 2004. *SBAT: A Tool for Estimating Metal Bioaccessability in Soils*, ORNL/TM-2004/49, Oak Ridge National Laboratory: Oak Ridge, TN.

Schroder, J.L., N.T. Basta, S.W. Casteel, T.J. Evans, M.E. Payton, and J. Si. 2004. *Validation of the in vitro gastrointestinal (IVG) method to estimate relative bioavailable lead in contaminated soils*. Journal of Environmental Quality. **33**(2): p. 513-521.

Basta, N., and R. Gradwohl. 2000. *Estimation of Cd, Pb, and Zn bioavailability in smelter- contaminated soils by a sequential extraction procedure*. Journal of Soil Contamination. **9**(2): p. 149-164.

Dayton, E.A. 2003. *Relative Contribution of soil properties to modifying the phytotoxicity ad bioaccumulation of cadmium, lead and zinc to lettuce*. Oklahoma State University, Stillwater, OK.

Webb, S.M. 2004. *SixPACK: Sam's interface for XAS Package*, Stanford, CA: Stanford Synchrotron Radiation Laboratory.

Ravel, B., and M. Newville. 2005. *ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT*. Journal of Synchrotron Radiation. **12**: p. 537-541.

Ankudinov, A.L., C.E. Bouldin, J.J. Rehr, J. Sims, and H. Hung. 2002. *Parallel calculation of electron multiple scattering using Lanczos algorithms*. Physical Review B. **65**(10).

Oday, P.A., J.J. Rehr, S.I. Zabinsky, and G.E. Brown. 1994. *Extended X-Ray-Absorption Fine-Structure (Exafs) Analysis of Disorder and Multiple-Scattering in Complex Crystalline Solids*. Journal of the American Chemical Society. **116**(7): p. 2938-2949.

Beaulieu, B.T., and K.S. Savage. 2005. *Arsenate adsorption structures on aluminum oxide and phyllosilicate mineral sufaces in smelter-impacted soils*. Environmental Science & Technology. **39**(10): p. 3571-3579.

Webb, S.M. *Sam's Microprobe Analysis Kit (SMAK) v. 0.372006*, Stanford, CA: Stanford Synchrotron Radiation Laboratory.

Peterson, M.L., G.E. Brown, G.A. Parks, and C.L. Stein. 1997. *Differential redox and sorption of Cr(III/VI) on natural silicate and oxide minerals: EXAFS and XANES results*. Geochimica Et Cosmochimica Acta. **61**(16): p. 3399-3412.

ASTM. *Standard guide for conducting a laboratory soil toxicity or bioaccumulation tests with the Lumbricid earthworm Eisenia foetida*, in E 1676-971999.

ASTM International. 2004. *Standard Guide for Conducting Soil Laboratory Toxicity or Bioaccumulation Tests with the Lumbricid Earthworm Eisenia fetida and the Enchytraeid Potworm Enchytraeus albidus*. E 1676-04. **11.04** (American Society for Testing and Materials, Philadelphia PA).

Weis, C.a.L., J.M. 1991. *Characteristics to consider when choosing an animal model for the study of lead bioavailability*. Chemical Speciation Bioavailability. **3**: p. 113-119.

Drexler, J.W. and W.J. Brattin, *An in vitro procedure for estimation of lead relative bioavailability: With validation*. Human and Ecological Risk Assessment, 2007. **13**(2): p. 383-401.

Juhasz, A.L., J. Weber, E. Smith, R. Naidu, M. Rees, A. Rofe, T. Kuchel, and L. Sansom. 2009. *Assessment of Four Commonly Employed in Vitro Arsenic Bioaccessibility Assays for Predicting in Vivo Relative Arsenic Bioavailability in Contaminated Soils*. Environmental Science & Technology. **43**(24): p. 9487-9494.

Whitacre, S.D. 2009. *Soil controls on arsenic bioaccessibility: arsenic fractions and soil properties*. M.S. Thesis. The Ohio State University: Columbus, OH.

Bang, J.S., and D. Hesterberg. 2004. *Dissolution of trace element contaminants from two coastal plain soils as affected by pH*. Journal of Environmental Quality. **33**(3): p. 891-901.

APPENDIX A

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